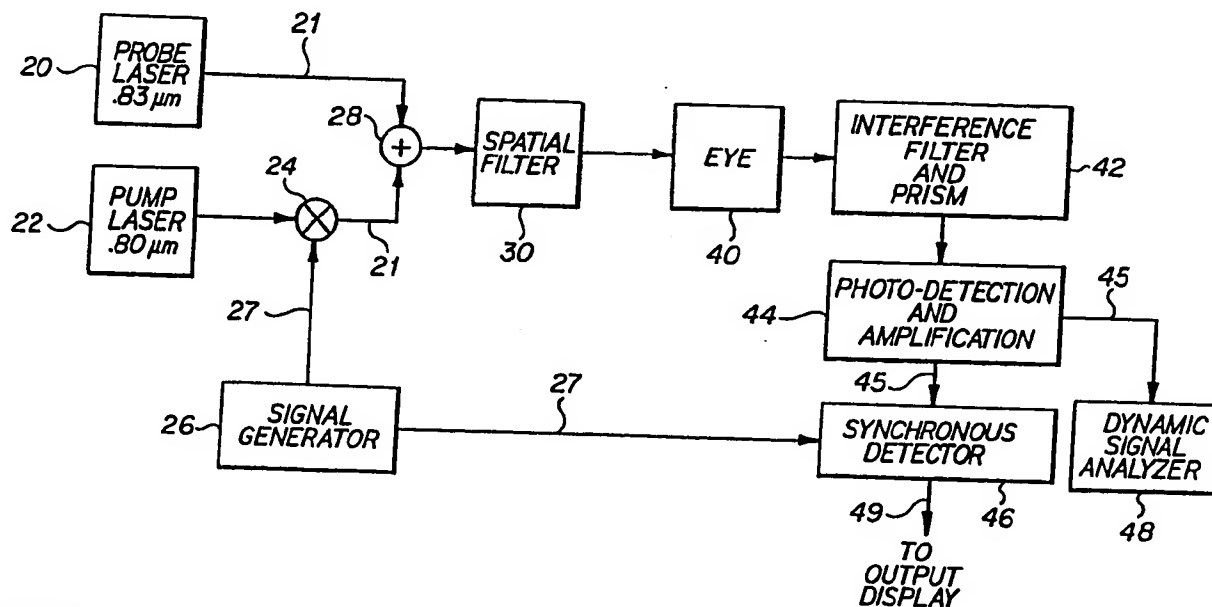




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(54) Title: NON-INVASIVE BLOOD GLUCOSE MEASUREMENT SYSTEM**(57) Abstract**

Stimulated Raman spectroscopy is used to non-invasively measure the concentration of a Raman active molecule, preferably D-glucose in the ocular aqueous humor of living being. The apparatus and method make use of two monochromatic laser beams, a pump laser beam and a probe laser beam. The output power of the pump laser beam is amplitude modulated, combined with the probe laser beam and directed into the ocular aqueous humor. The introduction of the laser beams into the ocular aqueous humor induces scattered Raman radiation, which causes a portion of the energy at the pump frequency to shift over to the probe frequency. The pump and probe laser beams are then detected as they exit the ocular aqueous humor. The probe laser beam is filtered, converted into an electrical signal and amplified. It is then compared to the modulation signal to generate an electrical signal representative of the concentration of D-glucose in the ocular aqueous humor.

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NON-INVASIVE BLOOD GLUCOSE MEASUREMENT SYSTEM**BACKGROUND OF THE INVENTION****A. Field of the Invention**

The present invention relates to a non-invasive method
5 and apparatus for measuring the concentration of D-glucose
in the ocular aqueous humor. More particularly, the
present invention is a non-invasive technique for the
in vivo measurement of the glucose concentration in the
ocular aqueous humor employing the stimulated Raman
10 effect.

B. Background of the Invention

Diabetes Mellitus is a major health problem in the
world today because of the physical complications which
arise from living many years with above-normal blood
15 glucose levels. Currently, over 11 million people suffer
from diabetes in the United States alone. The two most
common forms of diabetes are Type I, juvenile-onset, and
Type II, adult-onset. Type I diabetes destroys the vast
majority of the insulin-producing beta cells in the
20 pancreas, forcing its sufferers to take multiple daily
insulin injections. Type II diabetes is usually less
severe than Type I as some endogenous insulin production
still occurs and, as a result, Type II diabetes can often
be controlled by diet alone.

25 The body requires insulin for many metabolic
processes; it is particularly important to the metabolism
of glucose. It is believed that many of the physical
complications associated with diabetes could be avoided if
normal blood glucose levels were maintained throughout
30 each day. A diabetic's blood glucose level can fluctuate
widely around each meal. Maintaining normal blood glucose
levels and reducing these fluctuations requires using some
form of feedback to regulate the multiple daily insulin
shots of Type I diabetics or the diet of Type II
35 diabetics.

Currently, the blood glucose level can be determined

by a chemical reaction performed on a blood sample. Although the state of the art glucose measurement devices are very accurate, the need for a blood sample for each measurement limits their utility. The most dedicated diabetic patient may take only 4 or 5 measurements per day, and many diabetics perform even fewer. Because a diabetic's blood glucose level can fluctuate by a factor of two or more in a period of an hour, this method cannot provide the feedback necessary to maintain a normal blood glucose level throughout the day.

A non-invasive blood glucose measurement technique would allow a large number of daily measurements to be taken without the problems associated with taking blood samples. Various schemes have been attempted to non-invasively measure blood glucose level. Many promising techniques attempt to measure the glucose level in the ocular aqueous humor because it has been shown that the ocular glucose level directly correlates to the blood glucose level and because the ocular aqueous humor provides a much simpler spectroscopic environment than the blood.

D-glucose occurs normally and in abundance in both the blood and the ocular aqueous humor. There are two anomers of D-glucose found in nature: α -D-glucose and β -D-glucose, which differ only in the orientation of the groups attached to the C-1 carbon. Physically, these two anomers of D-glucose can be distinguished by their optical activity; i.e. based upon their ability to rotate the plane of polarization when illuminated with plane polarized light. In general, the specific rotation, $[\alpha]$, is defined as

$$(1) \quad [\alpha] = \frac{\alpha}{ld}$$

where α is the total optical rotation of the plane of polarization measured in degrees, l is the length of the sample in decimeters, and d is the density in g/cm^3 . The specific rotations of α -D-glucose and β -D-glucose are 112.5 and 19 degrees, respectively. In solution, one anomer is converted into the other as necessary to achieve an equilibrium solution which has a specific rotation of 52.7 degrees.

Since the specific rotation of D-glucose in solution is known, from Equation (1) one can infer the concentration of D-glucose in a given sample by measuring the total optical rotation. The accuracy and linearity observed at very low D-glucose concentrations led March et al. to attempt non-invasive measurements in the eyes of 15 rabbits. See Rabinovitch, March and Adams, Non-invasive Glucose Monitoring of the Aqueous Humor of the Eye: Part I. Measurements of Very Small Optical Rotations, 5 Diabetes Care 1254 (May-June 1982); March, Rabinovitch and Adams, Non-invasive Glucose Monitoring of the Aqueous
20 Humor of the Eye: Part II. Animal Studies and the Scleral Lens, 5 Diabetes Care 259 (May-June 1982). Unfortunately, March and his colleagues experienced great difficulty in measuring the concentration of D-glucose in the ocular aqueous humor. Many compounds in the ocular aqueous humor
25 other than D-glucose are optically active and contribute to the rotation of the plane of polarization. In addition, the cornea has birefringence, which causes a further rotation of the plane of polarization of the incident light. See generally, Gough, The Composition of
30 and Optical Rotary Dispersion of Bovine Aqueous Humour, 5 Diabetes Care 266 (May-June 1982).

Both spontaneous and stimulated Raman spectroscopy are potentially useful to measure the concentration of a Raman active molecule in a medium. With spontaneous Raman
35 spectroscopy a monochromatic laser beam is directed into a

Raman-active medium. Some of the incident beam is transmitted, some of it is absorbed, and some of it is scattered. A small fraction of the radiation scattered is shifted in frequency from the incident beam. The amount of this relative frequency shift is related to the vibrational states of the Raman active molecules in the medium. The problem with spontaneous Raman scattering is that the Raman power is scattered in all directions. This makes the detection of the scattered radiation difficult for in vivo measurements.

Stimulated Raman spectroscopy (SRS) directs two monochromatic laser beams, a pump laser beam and a probe laser beam, into a Raman active medium. If the power of the pump laser is modulated, then the spontaneous Raman scattered power will also be modulated, which will induce a signal on the probe laser beam. Thus, rather than measuring the spontaneous Raman scattered power directly, a measurement of an intensity fluctuation of the probe laser beam can be made.

Stimulated Raman spectroscopy has been successfully used to measure very low concentrations of certain selected organic liquids diluted by water and other solvents. Owyong and Jones performed a series of experiments with benzene using stimulated Raman scattering techniques. See Owyong, Sensitivity Limitations for CW Stimulated Raman Spectroscopy, 22 Optics Communications 323 (Sept. 1977); Owyong and Jones, Stimulated Raman Spectroscopy Using Low-Power CW Lasers, 1 Optics Letters 152 (November 1977). Their experimental set-up consisted of two lasers, a tunable pump laser and a fixed frequency probe laser. The pump laser power was modulated while the probe laser power was held constant. The two laser beams were combined and focused through a benzene cell. In the cell the stimulated Raman effect caused a very small fraction of the power at the pump wavelength to be shifted

to the probe wavelength. Thus, at the output of the benzene cell the probe laser beam carried a small modulation signal whose amplitude was directly proportional to the concentration of the benzene in the cell. The probe wavelength was separated from the pump and converted to an electrical signal by a photodiode. Both the probe signal and the input pump modulation signal were fed into a synchronous detector which greatly improved the signal-to-noise ratio. The pump laser is then repeatedly tuned to new wavelengths to scan a range of wavelengths, thus, obtaining a Raman spectra for the Raman-active liquid or gas. This is the same type of spectrum obtainable by using a commercially available Raman spectrometer.

15 Until the present invention, no one has developed a technique which would allow for non-invasive in vivo measurement of the glucose concentration in the ocular aqueous humor. March attempted a non-invasive technique employing an energy wave transmitter, such as an infrared source located on one side of the cornea and an associated detector on the opposite side of the cornea. See U.S. Patent 3,958,650. The wave source is aimed to cause the radiation to pass through the cornea and the aqueous humor to the detector. A transmitter is mounted adjacent to the detector and coupled thereto for transmitting a signal that is a function of the radiation level detected. This technique is seriously flawed. The radiation detected will be a function of the concentration of all substituents in the humor, not just glucose. The later optical rotation technique of March, Rabinovitch and Adams suffers from a similar flaw. Further, no one, until now, has determined whether stimulated Raman spectroscopy may be successfully used to measure concentrations of glucose in the ocular aqueous humor.

SUMMARY OF THE INVENTION

A non-invasive blood glucose measurement technique would allow more frequent measurement of blood glucose concentrations without the problems associated with taking 5 blood samples. The present invention achieves this goal by providing an apparatus and a method for non-invasively measuring the in vivo concentration of an Raman active molecule in the ocular aqueous humor by using stimulated Raman spectroscopy. The apparatus of the present 10 invention includes a means for emitting a probe laser beam and a means for emitting a pump laser beam. Both means emit monochromatic laser light and are separated in wavelength by a wavelength chosen to be within a characteristic Raman shift spectrum for the Raman active 15 molecule. By setting the separation in wavelength between the pump and probe lasers, one may select which one of a number of Raman active molecules will be measured. In the preferred embodiment the selected Raman active molecule is D-glucose and the separation between the probe wavelength 20 and pump wavelength is chosen to be 518 cm^{-1} in accordance with the characteristic Raman shift spectrum for D-glucose.

The apparatus also includes a modulating means for modulating the output power of the pump laser beam. A 25 power source is provided for the probe laser for maintaining its power output substantially constant. The modulated pump laser beam is then combined with the probe laser beam by a means for directing the combined laser beams into the ocular aqueous humor.

30 The introduction of light into the ocular aqueous humor stimulates Raman radiation which shifts energy from the pump frequency to the probe frequency, thereby inducing fluctuations in the probe laser beam directly related to the concentration of the selected Raman active 35 molecule in the ocular aqueous humor. After the probe

laser beam exits the ocular aqueous humor, means are provided for detecting the probe laser beam and converting it into a Raman electrical signal. The Raman electrical signal is then compared to the modulation signal by a synchronous detector, a dynamic signal analyzer, or a computer based synchronous detection system, to produce a voltage representative of the concentration of the Raman active molecule in the ocular aqueous humor.

The method of the present invention non-invasively measures the in vivo concentration of an Raman active molecule in the ocular aqueous humor using stimulated Raman spectroscopy. Two monochromatic laser beams are provided, a probe laser beam and a pump laser beam. The probe laser beam and the pump laser beam wavelengths are separated from each other by a wavelength within a characteristic Raman spectrum for the Raman active molecule being measured. In the preferred embodiment, the Raman active molecule is D-glucose and the separation in frequency preferably chosen to be 518 cm^{-1} . The output power of the probe laser beam should be maintained substantially constant, while the output power of the pump laser beam is modulated by a modulation signal. The probe laser beam and the modulated pump laser beam are combined and directed into the ocular aqueous humor, thereby stimulating Raman scattered radiation. The probe laser beam is detected after it exits the ocular aqueous humor and converted into an electrical signal. The electrical signal is then compared with the modulation signal to produce a voltage representative concentration of the Raman active molecule.

It is, therefore, an object of the present invention to provide a system for measuring an Raman active molecule in the ocular aqueous humor.

It is another object of the present invention to provide a system for measuring very small D-glucose concentrations.

It is yet another object of the present invention to make non-invasive in vivo measurements of D-glucose concentration.

It is a further object of the present invention to
5 allow multiple daily measurements of D-glucose to be made non-invasively.

It is a still further object of the present invention to provide a non-invasive glucose measurement system which is inexpensive to manufacture, durable in construction,
10 and efficient in operation.

These and other advantages will become apparent in the discussion below.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph of the spontaneous Raman spectrum
15 for D-glucose.

Fig. 2 illustrates the stimulated Raman spectroscopy (SRS) wavelength selection for the preferred embodiment.

Fig. 3 is a block diagram of one embodiment of the present invention.

20 Fig. 4 depicts the preferred amplitude modulation of the pump laser power versus time.

Fig. 5 is a top view of an eye showing entering and exiting laser beams.

Fig. 6 illustrates the modulation of the detected
25 probe laser power after extraction versus time.

Fig. 7 illustrates the apparatus of the present invention for in vivo measurement.

Fig. 8 is a block diagram an alternative embodiment of the present invention.

30 Fig. 9 is a block diagram of another alternate embodiment of the present invention using bulk optics.

DETAILED DESCRIPTION

When a monochromatic laser beam is incident on a Raman-active medium some of the incident beam is
35 transmitted, some of it is absorbed, and some of it is

scattered. A small fraction of the radiation scattered is shifted in frequency from the incident beam. The amount of this relative frequency shift is related to the vibrational states of the molecules in the medium. The D-glucose molecule has several possible Raman active vibrational states so that the Raman scattered power forms a spectrum which is characteristic of D-glucose alone.

Turning now the drawings in which like numerals denote corresponding parts the preferred embodiment of the present invention is shown. Fig. 1 illustrates the characteristic spectrum for D-glucose dissolved in water showing the relative intensity of spontaneous Raman power versus the frequency shift. Each of the peaks in the spectrum corresponds to a particular vibration of the D-glucose molecule, the largest peak occurring at a frequency shift of 518 cm^{-1} . The absolute intensities of the peaks are directly related to the concentration of the Raman-active molecule, in this case D-glucose.

Use of a single monochromatic laser beam to generate spontaneous Raman scattering is difficult for in vivo measurements since the Raman power is scattered in all directions. This problem can be solved by having two monochromatic laser beams (a pump laser and a probe laser) incident on the chosen sample. Thus, in the preferred embodiment, the probe laser is at the same frequency as the Raman scattered power from a large peak in the D-glucose spectrum, as illustrated in Fig. 2. The pump laser is at a frequency whose difference from the probe frequency is equal to the frequency shift of the large peak selected for the probe laser. In order to use stimulated Raman spectroscopy to measure the concentration of D-glucose in the ocular aqueous humor, the frequency difference between the pump laser beam and the probe laser beam must be chosen to coincide with one of the peaks in the Raman spectrum for D-glucose. If the power output of

the pump laser is modulated, then the spontaneous Raman scattered power will also be modulated which will induce a signal on the probe laser beam. Rather than measuring the spontaneous Raman scattered power directly, a measurement of an intensity fluctuation on the probe laser beam is made. This method is called stimulated Raman spectroscopy.

As shown in Fig. 3, the present invention involves two lasers, a probe laser 20 and a pump laser 22. Both lasers 20 and 22 emit monochromatic laser beams. The relative wavelength difference between these two laser beams is adjusted to be the same as the wavelength shift of one of the largest spontaneous Raman peaks for D-glucose, 518 cm^{-1} . Other peaks unique to the D-glucose spectrum may be chosen, for example, 400 cm^{-1} .

The probe laser 20 chosen operates at a wavelength of approximately 0.83 micrometers with an output power of 20 mW. The probe laser power output should remain substantially constant over time in order to minimize errors in measurement. Thus, the sensitivity of the system is directly related to the ability to maintain the probe laser power output constant. The laser diode chosen, an SDL-1401-H2, is slightly tunable with temperature.

The pump laser 22 chosen emits light with a wavelength of approximately 0.8 micrometers and an output power of 100 mW. The power output of the pump laser should ordinarily be greater than that of the probe laser by about 5X depending upon the threshold conditions for the medium. The pump laser wavelength is also slightly tunable with temperature to allow fine adjustment for optimal signal level.

The actual wavelengths chosen for the pump and probe laser beams are not as important as the separation between them. That separation should correspond to a vibrational state of the Raman active molecule being measured. For

example, should it be desirable to concentrate on the D-glucose peak at 400 cm^{-1} , a different separation would be chosen for the wavelengths output by the probe and pump laws. The particular wavelengths of 0.8 and 0.83 were 5 selected because of the availability of commercial diode lasers of such wavelengths.

The pump laser beam is amplitude modulated by a biased square wave signal from signal generator 26. The output of signal generator 26 used to modulate the current to the 10 diode of the pump laser 22 thereby modulating the amplitude of the output of the laser beam. The output of pump laser 22 is a biased square wave, with a maximum amplitude of approximately 100 mW and a minimum of approximately 0 mW. An example of amplitude modulated 15 pump laser power over time is illustrated in Fig. 4.

It is possible to use other types of modulation to measure the concentration of Raman active molecules using stimulated Raman spectroscopy. Amplitude modulation was chosen over other types of modulation, such as pulse width 20 modulation, because amplitude modulation is easier to generate. Further, the type of modulation chosen affects the complexity of the detection scheme that must be used. The choice of modulation technique also affects the power incident upon the eye. Any technique which reduces this 25 power reduces possible damage to the eye and is to be preferred.

The probe laser beam and the amplitude modulated pump laser beam are fed to a fiber optic coupler 28 via fiber optic pigtails 21. Only 50% of the power in the probe and 30 pump laser beams is coupled into the fiber optic pigtails 21 due to their coupling efficiency. Another 50% of the power in the probe and pump laser beams is lost when the fiber optic pigtails 21 are joined together by optic coupler 28. Thus, the maximum power which could reach eye 35 40 is approximately 25 mW when starting with a laser power

output of 100 mW.

Fiber optic coupler 28 combines the probe and modulated pump laser beams and directs them into an optional spatial filter 30. The spatial filter 30 converts the cross-section intensity to a Gaussian Distribution which allows the beam to be focused more precisely and insures the complete combination of the two wavelengths before the laser beams are directed into the eye 40.

10 The combined laser beams travel through fiber optic cables associated with means for delivering the laser beams to the eye, preferably in the form of a handset (not shown). The handset is held up against eye 40 and directs the probe and modulated pump laser beams into the ocular
15 aqueous humor. The trajectory of incident laser beams 60 can be seen in Fig. 5. The laser beams 60 are passed through the cornea 36 and the aqueous humor 38 in such a manner as to bypass the lens 32 and the iris 34. Pump and probe beams 60 excite stimulated Raman radiation while
20 inside the ocular aqueous humor 38. The scattered Raman radiation causes a small amount of the energy at the pump frequency to be shifted to the probe frequency, thereby inducing fluctuations in the previously constant power level of the probe laser beam, as illustrated in Fig. 6.
25 These fluctuations in the probe laser beam power are directly related to the concentration of D-glucose in the ocular aqueous humor 38. The now modulated probe laser beam and the pump laser beam exit the eye and are coupled into an optical fiber in the handset-cable assembly.

30 The coupled detected laser beams are passed through a set of cascaded narrow band interference filters 42. These optional filters are centered at the probe wavelength plus or minus about 5 nm, thereby filtering the pump laser beam away from the probe laser beam. Thus, if
35 the probe wavelength is set at 0.83 microns, the band

width (BW) for the filters would be $BW = 825 \text{ nm} < \lambda < 835 \text{ nm}$. The filters are cascaded because the desired reduction in the pump laser power cannot be achieved with a single filter. Further reduction in the power of the 5 pump wavelength may be accomplished with a prism or grating.

Thereafter, the modulated probe laser beam is applied to a photo-voltaic diode in photodetector/amplifier 44, which outputs an electrical signal. The photo-voltaic 10 diode provides a current output in relation to the amount of light input. The photodetector/amplifier 44 includes a low-noise amplifier which amplifies the low current level output of the photo-voltaic diode to achieve a voltage level compatible with the circuitry of the synchronous 15 detector 46. A transresistance gain of greater than 10^8 is achieved by the low noise amplifier, which also filters off the large DC bias.

The operating band of the low noise amplifier is determined by the noise spectrum of the probe laser. The 20 noise spectrum of the probe laser is relatively constant between 1-10 KHZ, and increases below this frequency range. Restricting the passband of the low noise amplifier to this frequency range helps eliminate undesired noise from the probe laser. Those skilled in 25 the art will understand that the passband chosen depends upon the noise spectrum particular to the laser used as the probe laser. Different lasers may be expected to require different low noise amplifiers.

The output 45 of the photodetector/amplifier 44 is fed 30 to synchronous detector 46, such as a Princeton HR-8 PAR lock-in amplifier. The synchronous detector 46 compares output 45 to the output 27 of signal generator 26, and generates signal 49, which is representative of the concentration of D-glucose in the ocular aqueous humor. 35 The synchronous detector output 49 may then be fed to any

standard display element.

Use of the lock-in amplifier involves using the pump laser modulation signal as an external reference signal and feeding the SRS signal into the signal input of the 5 amplifier. The bandpass filter for the external reference may need to be tuned to the pump modulation frequency depending on the model of the lock-in amp. A phase offset between the reference and the SRS signal should be zeroed as indicated in the installation manual. Then the 10 appropriate gain setting for the SRS input signal should be set. The time constant or integration time setting may vary depending upon the noise present on the signal. The DC output signal may be read from the meter in the unit or directed to an external display.

15 An alternative, and more costly, method of generating a representative electrical signal from the photodetector/amplifier 44 output is to feed output 45 to a dynamic signal analyzer 48, such as an HP 3561 made by Hewlett-Packard. Analyzer 48 measures the power contained 20 in the detected probe laser beam at the modulation frequency. This power is likewise related to the D-glucose concentration. Thus, it is possible to use at least two independent methods to calculate the D-glucose concentration.

25 The procedure for the dynamic signal analyzer involves connecting the SRS signal to the input jack of the analyzer. The soft key programming is generally discussed in the user's manual for the analyzer. The frequency span should be set to a center frequency equal the pump 30 modulation frequency and a span of about 100 Hz which may vary depending upon signal noise. Preferably, the unit should also be programmed to RMS average 50 samples. There is a setting for peak tracking which will display a numerical value for the frequency peak in the local 35 portion of the signal spectrum which is currently

displayed. The vertical scale units should be set to "linear" and thus the value for the SRS signal corresponding to the glucose concentration will be the value of the peak frequency component. The numerical 5 value is displayed on the screen of the analyzer.

Before using the apparatus of the present invention background measurements should be made to establish a signal reference point and to be certain that there are no spurious signals in the passband of the low noise 10 amplifier which forms part of the photodetector/amplifier 44. A noise spectrum measured by the synchronous detector 46 with only the probe laser turned on should be made. Another important background measurement is pump laser power "leakthrough". Although the two narrow band 15 interference filters filter the pump wavelength, some small amount of pump power still reaches the detector 46. Since this signal is present whenever the pump laser is on, it will offset other measurements. In addition to the presence of pump leakthrough, stimulated Raman scattering 20 takes place in the fiber optic cables 21 which carry the input power to the eye 40 and this SRS signal offsets the SRS signal from D-glucose in the ocular aqueous humor. These offset signals do not directly limit the sensitivity of the D-glucose measurement since the SRS signal from the 25 D-glucose adds to the offset signals and thus the offset signals can be subtracted out in the calculation of the D-glucose concentration. But the relative amplitude of these offset signals does limit the detector gain which ultimately limits the system sensitivity.

30 Occurring naturally in the ocular aqueous humor, water is also a Raman-active molecule. There is no peak present in the Raman spectrum for water at the frequency shift of 518 cm^{-1} ; even so, some broad features of the water spectrum will produce an SRS signal at a shift of 35 518 cm^{-1} . This SRS signal from water contributes to the

offset signal and should be subtracted out in the calculation of D-glucose concentration.

A schematic of the present invention for in vitro measurement can be seen in Fig. 7. The entire apparatus is mounted on a wheeled cart 68. The pump laser (SDL-2412-H2) and its power supply 72 are mounted vertically on one side of the cart, while the probe laser (SDL-2412-H2) and its power supply 70 are mounted vertically on the opposite side of the cart. The preferred power supply for the probe laser is an LDX 3620 by ILX lightwave, Montana, because of its low noise current to the laser diode and constant power mode having an automatic compensation feature. Outputs from each of the pump and probe lasers are fed by optical fiber pigtails 73 to a fiber optic coupler 75. The pigtails are multi-mode fiber optic cables to match those supplied with the lasers. The combined probe and pump laser beams are then fed by fiber optic cables 76 from the fiber optic coupler 75 up to the spatial filter 77 and from there into an eye, or alternatively, into a glucose test cell 78, shown in place on a table behind the cart. The spatial filter 77 is preferably a Model 900 from Newport Optics. Glucose test cells 78 are used for in vitro measurement. The test cell 78 is machined plastic with special windows of a high quality, low impurity glass with a special coating to reduce reflections of optical wavelengths selected for the lasers. Preferred coatings include a magnesium fluoride or a coating broadband near infrared coating like Newport #AR.16.

A set of interference filters 79, previously described, receive the pump and probe laser beams as they exit the glucose cell 78. Fiber optic cable 81 passes the detected laser beams from filters 79 to detector unit 74, which houses both the photo-voltaic diode and the low noise amplifier. The output from the detector unit 74 is

then fed to the synchronous detection unit. A switchable laser temperature readout 82 may be provided to monitor the output of lasers 70, 72.

A block diagram of an alternative embodiment of the present invention is shown in Fig. 8. The alternative apparatus incorporates both a probe laser 120 and a pump laser 122. These lasers are both monochromatic and both operate at the same wavelengths discussed hereinabove with respect to the preferred embodiment of Fig. 3. The pump laser 122 is modulated using an AM modulation source 126, such as HP 3314A.

The output of probe laser 120 is connected to an optical coupler 150 which is reversed to split the probe laser output into two beams, one containing 5% and the other containing 95% of the probe laser power.

Another fiber optic coupler 128 combines ninety-five percent of the probe laser power with the modulated pump laser beam and outputs the combined beams to spatial filter 130. Using a handset (not shown) the combined beams are then directed into the eye 140. Inside the ocular aqueous humor of the eye 140 stimulated Raman radiation causes a portion of the power at the pump frequency to be shifted to the probe frequency, thereby modulating the probe laser beam. As the pump probe beams exit the eye they are coupled into a fiber optic cable to transport the SRS optical signal to the photo detector. The pump laser beam is filtered from the probe laser beam using a series of narrow band filters incorporated into the photodetector/amplifier 144. The modulated pump probe laser beam is then transduced from an optical signal into an electrical signal using a photo-voltaic diode. The electrical output from this diode is thereafter amplified using an extremely large gain, low noise amplifier incorporated in photodetector/amplifier 144 to achieve the signal levels compatible with a detection scheme. The

amplifier's gain is on the order of 10^8 . The amplifier's passband corresponds to a frequency range in which the probe laser's noise spectrum is substantially constant. The output of the photodetector/amplifier 144 is then fed 5 into a computer-based synchronous detector 156.

Five percent of the probe laser power is applied to the photodetector/amplifier 152, which is substantially similar to photodetector and amplifier 144 and which generates an electrical signal representative of the probe 10 laser beam power. This output may then be fed to an optional time delay 154 to compensate for the delay of the laser beam through the spatial filter, fiber optic coupler and photodetector amplifier path. However, since this time delay is small and is physically hard to realize, 15 time delay 154 may also be eliminated without substantially effecting the accuracy of the D-glucose measurement.

The computer-based synchronous detector 156 compares the photodetector/amplifier output 144 with the output of 20 the AM modulator 126 and photodetector/amplifier 152 output, allowing the computer-based synchronous detector 156 to compensate for amplitude variations in the probe laser beam caused by internal noise and thermal drift of the probe laser. A data acquisition/interface board to 25 the computer connects the signals from photodetector/amplifiers 144, 152 to the computer. The data acquisition/interface board preferably consists of 3 primary A/D channels. Two of these channels are 16-bit resolution and the third channel is only 8-bit 30 resolution. The 16-bit channels are used to convert the SRS signal and the PROBE Noise signal, while the 8-bit channel converts the modulation signal. The specifications should meet or exceed the following:

- 16-bit Converters - Analog Devices (1376A)
- 35 16-bit Track-Hold - Analog Devices (389KD)

Amplifiers

8-bit Converter - Analog Devices (574A)

8-bit Track-Hold - Analog Devices (HTC-0300)

A sampling rate of 10 KHz is used so the Nyquist frequency is 5 KHz. Appropriate anti-aliasing filters should be used prior to conversion which limit the bandwidth of the input signals to ≤ 5 KHz. Also there are four secondary A/D inputs to monitor various background activities like laser temperature. Once the analog signals are converted to digital values they are converted to floating point numbers and stored in memory arrays by low level programming. Currently the data is processed by algorithms to yield a stable SRS value for a given glucose concentration. These algorithms include the following:

1) The use of infinite impulse response (IIR) filters, such as a Butterworth filter, to further narrow the bandwidth of the signal.

2) The subtraction of amplitude noise originating on the PROBE Laser.

3) A conventional cross-correlation algorithm to yield a final result.

An alternate approach to the optical fiber band system described above would be to use entirely bulk optics in the system, thus eliminating the optical fibers completely. This bulk optics implementation, although more costly, will yield a higher system sensitivity than the optical fiber based system. This arises from the elimination of one of the sources of "leak through" signals and the conversion to single-mode laser diodes. Current technology limits the use of these high power single-mode laser diodes to a bulk optic system. These single-mode laser diodes concentrate their optical power into a much narrower spectral line which will greatly improve the SRS signal to noise ratio.

As illustrated in Fig. 9, the output of each laser

220,222 of the bulk optic system is delivered to beam collimating optics 221, 223, which preferably consist of a pair of cylindrical lenses for each laser, to focus the laser beam along their orthogonal axes to make a collimated beam from the asymmetrical cone shaped output of the lasers. Optical isolators 224, 225 are provided to prevent reflection of the beams back into the lasers. The isolators can be broad band isolators covering the band width of 750 to 950 nm, such as the Newport ISO-7885 optical isolator. The output of pump laser 222 passing through optical isolator 224 is delivered to an optical chopper 226 or electric optic modulator which preferably operates at 1kHz, though may be operated at different frequencies depending on the modulation desired for the pump laser output. Beam samplers 227, 260 are provided to reflect a small sample portion of the beam or beams, the amount of the sample depending upon the angle of placement of the beam sampler in the path of the beam. Beam sampler 227 is used to provide a small sample of the probe laser output to the photodetector and amplifier 252, the output of which 254 provides a probe laser noise signal to the computer based synchronous detection system 256. Beam sampler 260 provides a small sample of the beams provided to the spatial filter 230. The output of the photodetector and amplifier 262 coupled with the beam sampler 260 provides a synchronous detection reference signal 266 to the computer based synchronous system 256. An optical spectrum analyzer 264 is used to monitor the beams provided to the spatial filter 230 in the laboratory setting because the single mode lasers have a tendency to "mode hop" and change the wavelength of their output.

The rest of the path in Fig. 9 beginning with the spatial filter 230, the glucose sample 40 through to the computer based synchronous detection system 256 is the same as the embodiments discussed above with the

exception of the addition of the prism or grading 241. If a prism is used, a standard dispersing prism is preferred.

From the bulk optic system block diagram of Fig. 9 one can see that this system is functionally equivalent to the optical fiber based system. The major differences between the two implementations are as follows:

1) Now the polarization of the optical fields must be carefully controlled. It is vitally important that both the pump laser wavelength and the probe laser wavelength have nearly the same polarization of their optical fields. Here linear polarization is maintained throughout the system.

2) Due to technological limitations, the pump single-mode laser diode's optical power cannot be modulated by the modulation of its current. This is due to the fact that the wavelength of the laser diode's output power does depend upon its current. Thus, both laser diodes are operated with a constant current source. Now the pump laser's optical power is modulated by an optical chopper 226 which yields an equivalent optical power modulation to the optical fiber based system.

3) A key addition to this system is an optical isolator 224, 225 which is used with each laser diode to minimize reflections of the optical power back into the laser cavity. This is very important since the reflected power can cause the laser diode to change its wavelength during operation. This phenomenon has been observed in the optical fiber based system.

4) The spatial filter 230 is now required to insure that the optical power from each laser is collinearly focused through the glucose solution 240. The glucose solution may be in an optical test cell or in the ocular aqueous humor.

The addition of the optical spectrum analyzer 264 is

optional. Its purpose is to monitor a portion of the optical power to insure the proper optical wavelengths are present. Also the wavelengths for the single-mode laser diode have been changed slightly due to the availability 5 from the manufacturer. The difference between these two wavelengths still corresponds to a frequency difference of 518 cm^{-1} as previously discussed.

It will be obvious to those skilled in the art that many variations may be made in the embodiment chosen for 10 the purpose of illustrating the best mode of making and operating the present invention, without departing from the scope thereof as defined by the appended claims.

We claim:

1. A method of non-invasively measuring the concentration of a Raman active molecule in ocular aqueous humor using stimulated Raman spectroscopy, comprising the steps of:
 - generating a modulation signal;
 - emitting a probe laser beam having a first wavelength;
 - emitting a pump laser beam having a second wavelength differing from said first wavelength by a third wavelength selected to be within a characteristic Raman shift spectrum for the Raman active molecule;
 - modulating said pump laser beam using said modulation signal;
 - directing said probe laser beam and said modulated pump laser beam into the ocular aqueous humor thereby stimulating Raman scattered radiation, said Raman scattered radiation inducing fluctuations in said probe laser beam, said fluctuations being related to the concentration of the Raman active molecule in the ocular aqueous humor, said probe laser beam exiting the ocular aqueous humor;
 - detecting said probe laser beam after it exits the ocular aqueous humor;
 - converting said detected probe laser beam into a Raman electrical signal; and
 - producing a signal representative of the Raman active molecule in the ocular aqueous humor from said Raman electrical signal and said modulation signal.
2. The method of Claim 1 wherein said Raman active molecule is D-glucose.
3. The method of Claim 2 wherein said first wavelength is about 0.83 μm .
4. The method of Claim 3 wherein said second wavelength is about 0.8 μm .

5. The method of Claim 1 wherein the step of modulating said pump laser beam comprises amplitude modulating said pump laser beam.

6. The method of Claim 5 wherein said step of emitting the probe laser beam includes maintaining the power output of the probe laser beam substantially constant.

7. The method of Claim 1 further comprising the step of spatially filtering said probe laser beam and said modulated pump laser beam before said directing step.

8. The method of Claim 1 further comprising the step of amplifying said detected probe laser beam.

9. The method of Claim 1 wherein the step of producing a signal representative of the Raman active molecule includes digital signal analyzing the Raman electrical signal to produce an electrical signal representative of the concentration of the Raman active molecule.

10. The method of Claim 1 wherein the step of producing a signal representative of the Raman active molecule includes use of a synchronous detector to produce the signal representative of the Raman active molecule.

11. The method of Claim 1 wherein the power output of the pump laser beam is maintained at a higher level than the power output of the probe laser beam.

12. An apparatus for non-invasively measuring the concentration of a Raman active molecule in ocular aqueous humor using stimulated Raman spectroscopy, comprising:

- 5 means for generating a modulation signal;
 means for emitting a probe laser beam having a first wavelength;
 means for emitting a pump laser beam having a second wavelength differing from said first wavelength by
10 a third wavelength selected to be within a characteristic Raman shift spectrum for the Raman active molecule;
 modulating means for modulating said pump laser beam using said modulation signal;
 means for directing said probe laser beam and
15 said modulated pump laser beam into the ocular aqueous humor thereby stimulating Raman scattered radiation, said Raman scattered radiation inducing fluctuations in said probe laser beam said fluctuation being related to the concentration of the Raman active molecule in the ocular
20 aqueous humor; said probe laser beam exiting the ocular aqueous humor;
 means for detecting the probe laser beam after it exits the ocular aqueous humor;
 means for converting said detected probe laser
25 beam into a Raman electrical signal; and
 means for producing a signal representative of the Raman active molecule in the ocular aqueous humor from said modulation signal and said Raman electrical signal.

13. The apparatus of Claim 12 wherein said Raman
30 active molecule is D-glucose.

14. The apparatus of Claim 13 wherein said first wavelength is about 0.83 μ m.

15. The apparatus of Claim 14 wherein said second wavelength is about 0.8 μ m.

16. The apparatus of Claim 12 wherein said means for emitting said probe laser beam comprises a first monochromatic laser.

17. The apparatus of Claim 12 wherein said means
5 for emitting said pump laser beam comprises a second monochromatic laser.

18. The apparatus of Claim 12 wherein said means for directing includes a fiber optic coupler and fiber optic cable.

10 19. The apparatus of Claim 12 further comprising means for amplifying said detected probe laser beam.

20. The apparatus of Claim 12 further comprising means for digital signal analysis of said detected probe laser beam to produce an electrical signal representative
15 of the Raman active molecule.

21. The apparatus of Claim 12 wherein said means for detecting and said means for converting comprise a photodetector.

22. The apparatus of Claim 12 wherein said means
20 for producing said signal representative of the Raman active molecule comprises a synchronous detector.

23. The apparatus of Claim 12 wherein said means for producing said signal representative of said Raman active molecule comprises a dynamic signal analyzer.

24. The apparatus of Claim 12 wherein said means
25 for producing said signal representative of said Raman active molecule comprises a computer based synchronous detection system.

25. The apparatus of Claim 12 wherein the means for
30 emitting a probe laser includes a power supply for maintaining the output of the probe laser substantially constant.

26. The apparatus of Claim 12 wherein the signal
representative of said Raman active molecule is
35 representative of the concentration of said Raman active molecule.

27. An apparatus for non-invasively measuring the concentration of an Raman active molecule in the ocular aqueous humor, comprising:

a probe laser emitting a probe laser beam,
5 having a first wavelength;

a pump laser emitting a pump laser beam, having
a second wavelength which differs from said first
wavelength by a third wavelength selected to be within a
characteristic Raman shift spectrum for the Raman active
10 molecule;

a signal generator generating a modulation
signal;

a modulator receiving said modulation signal
and said pump laser beam;

15 a fiber optic coupler receiving said probe
laser beam and said modulated pump laser beam and
directing said laser beams into the ocular aqueous humor
thereby stimulating Raman scattered radiation, said Raman
radiation inducing fluctuations in said probe laser beam,
20 said fluctuations being related to the concentration of
the Raman active molecule in the ocular aqueous humor,
said probe laser beam exiting the ocular aqueous humor;

a photodetector receiving said probe laser beam
and producing an electrical signal; and

25 an amplifier developing a dc voltage
representative of the concentration of the Raman active
molecule in the ocular aqueous humor from said electrical
signal and said modulation signal.

28. The apparatus of Claim 22 wherein said Raman
30 active molecule is D-glucose.

29. The apparatus of Claim 23 wherein said
wavelength is about 0.83 μ m.

30. The apparatus of Claim 24 wherein said second
wavelength of about 0.8 μ m.

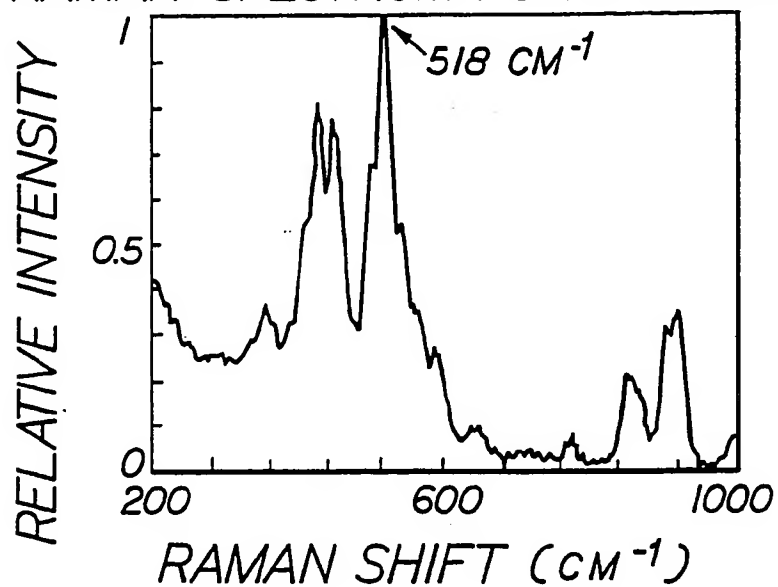
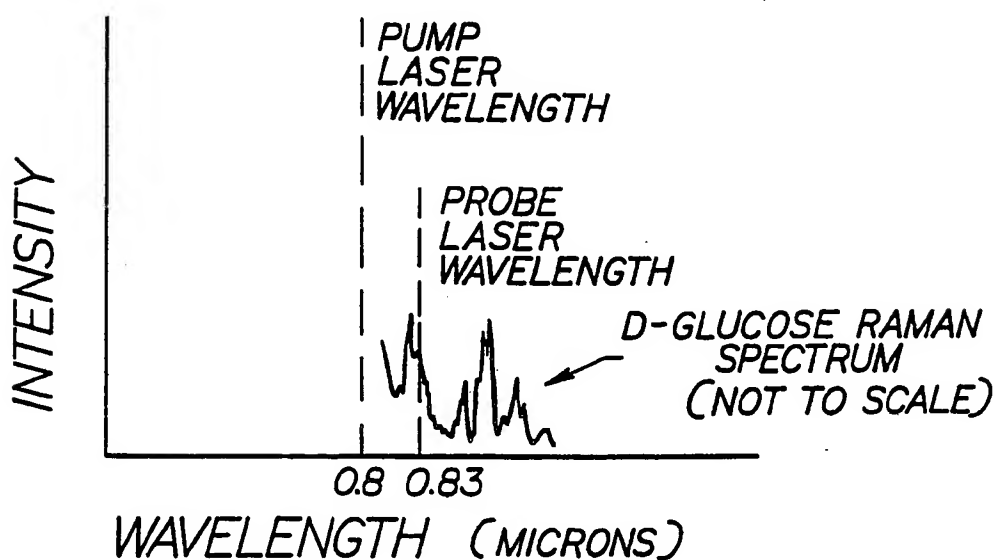
31. The apparatus of Claim 22 wherein said modulator comprises an amplitude modulator.

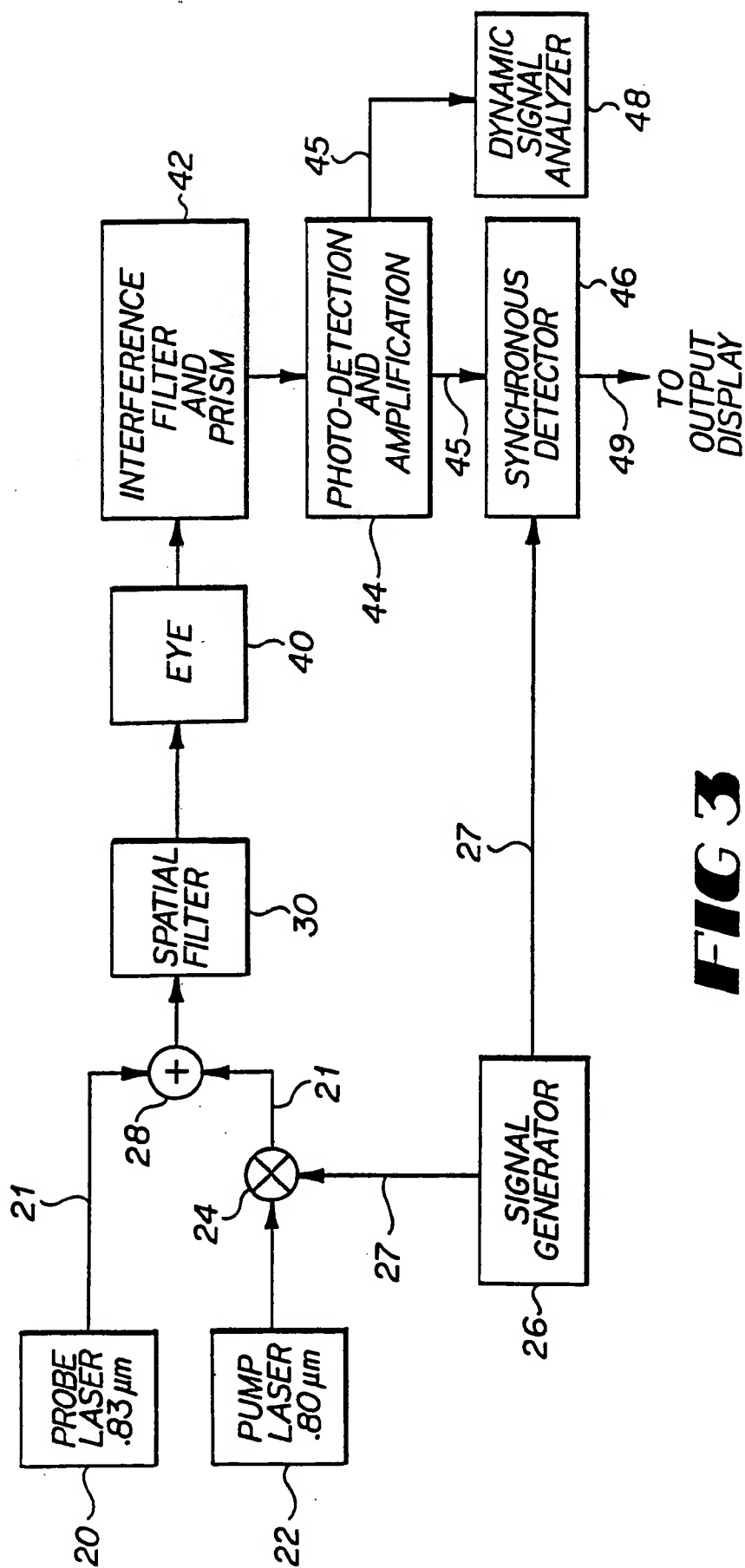
32. The apparatus of Claim 26 wherein said probe laser emits said probe laser beam having a substantially constant amplitude.

33. The apparatus of Claim 27 wherein said detector comprises a synchronous detector.

34. The apparatus of Claim 22 wherein said detector comprises a dynamic signal analyzer.

RAMAN SPECTRUM FOR D-GLUCOSE

**FIG 1****FIG 2**

**FIG 3**

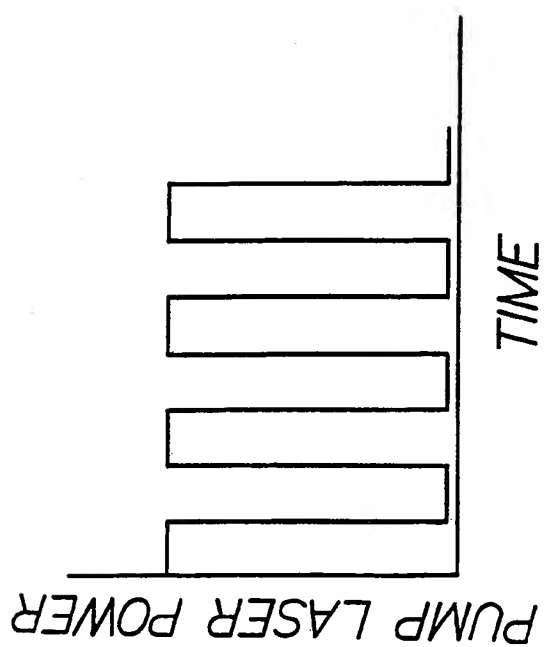


FIG 4

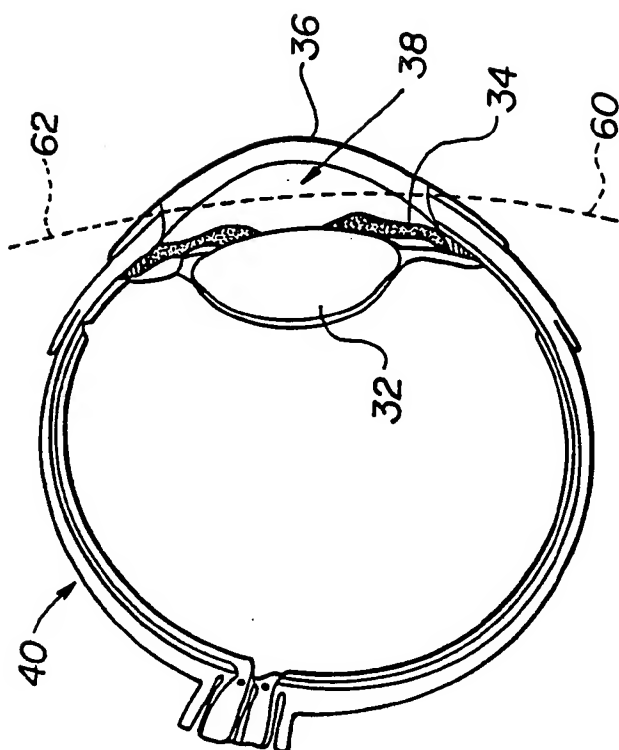
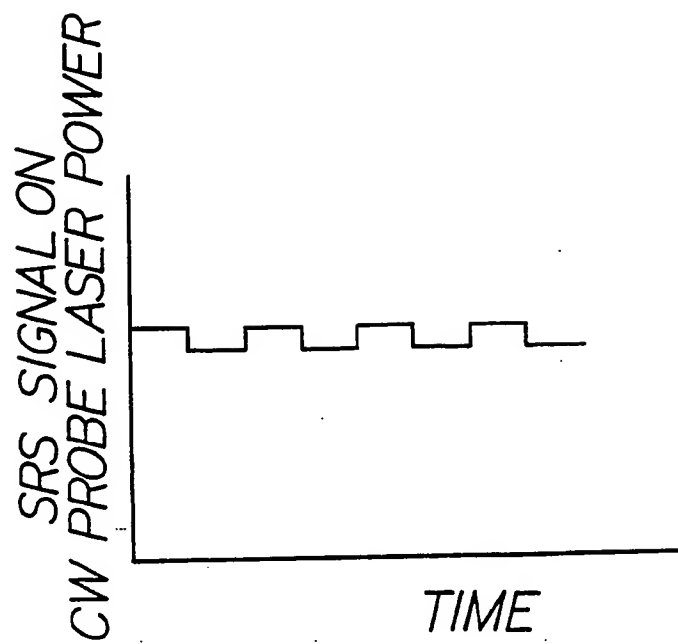
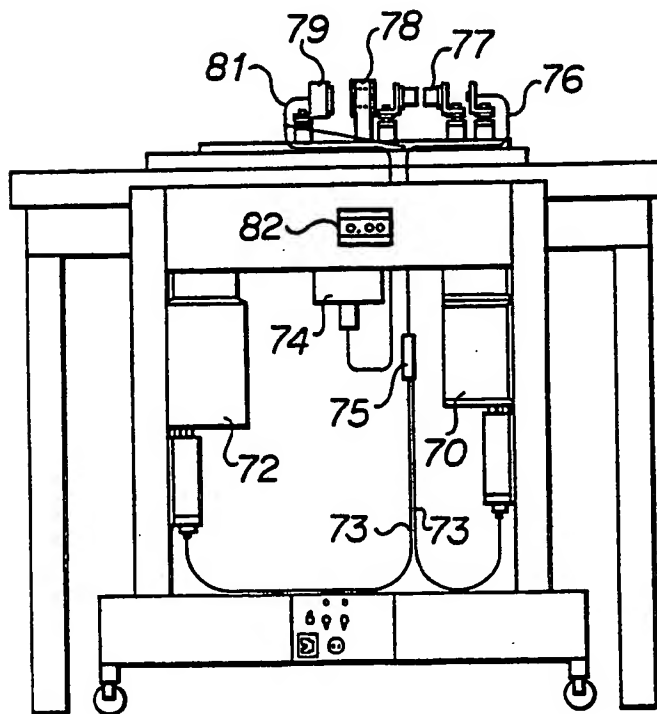
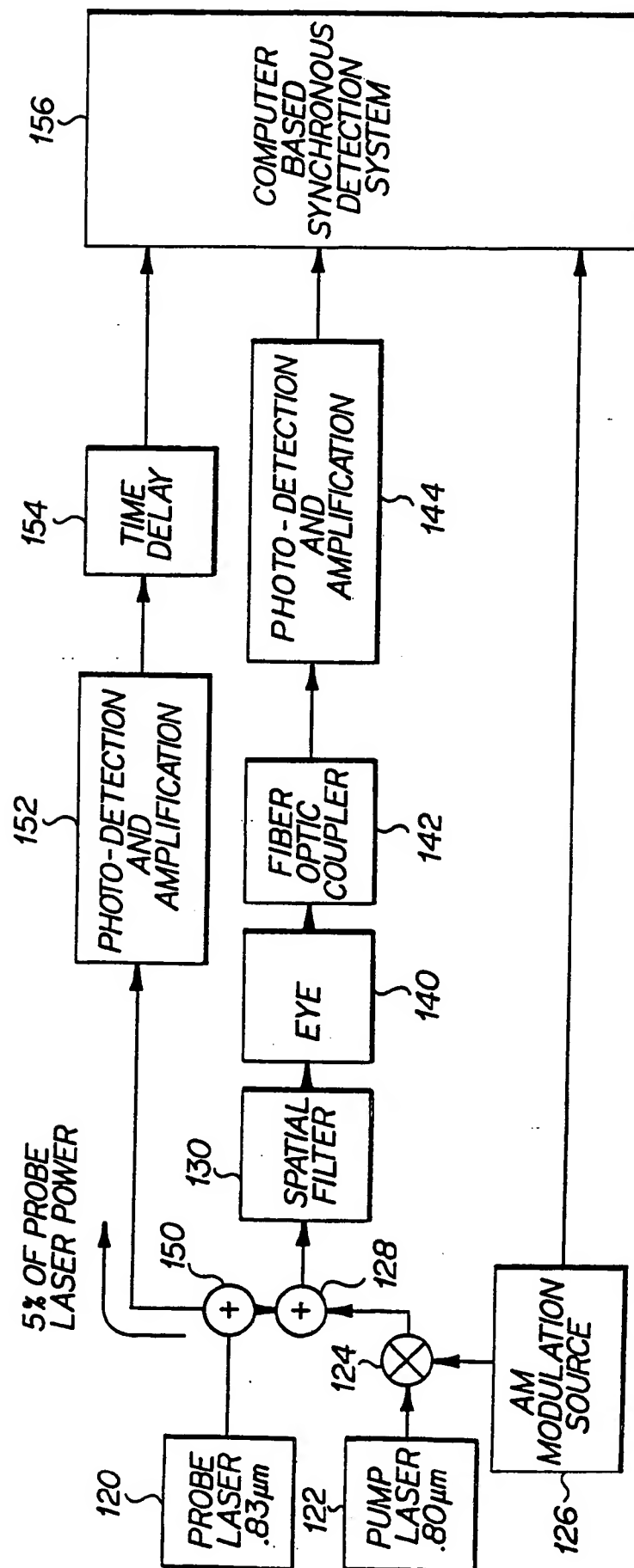
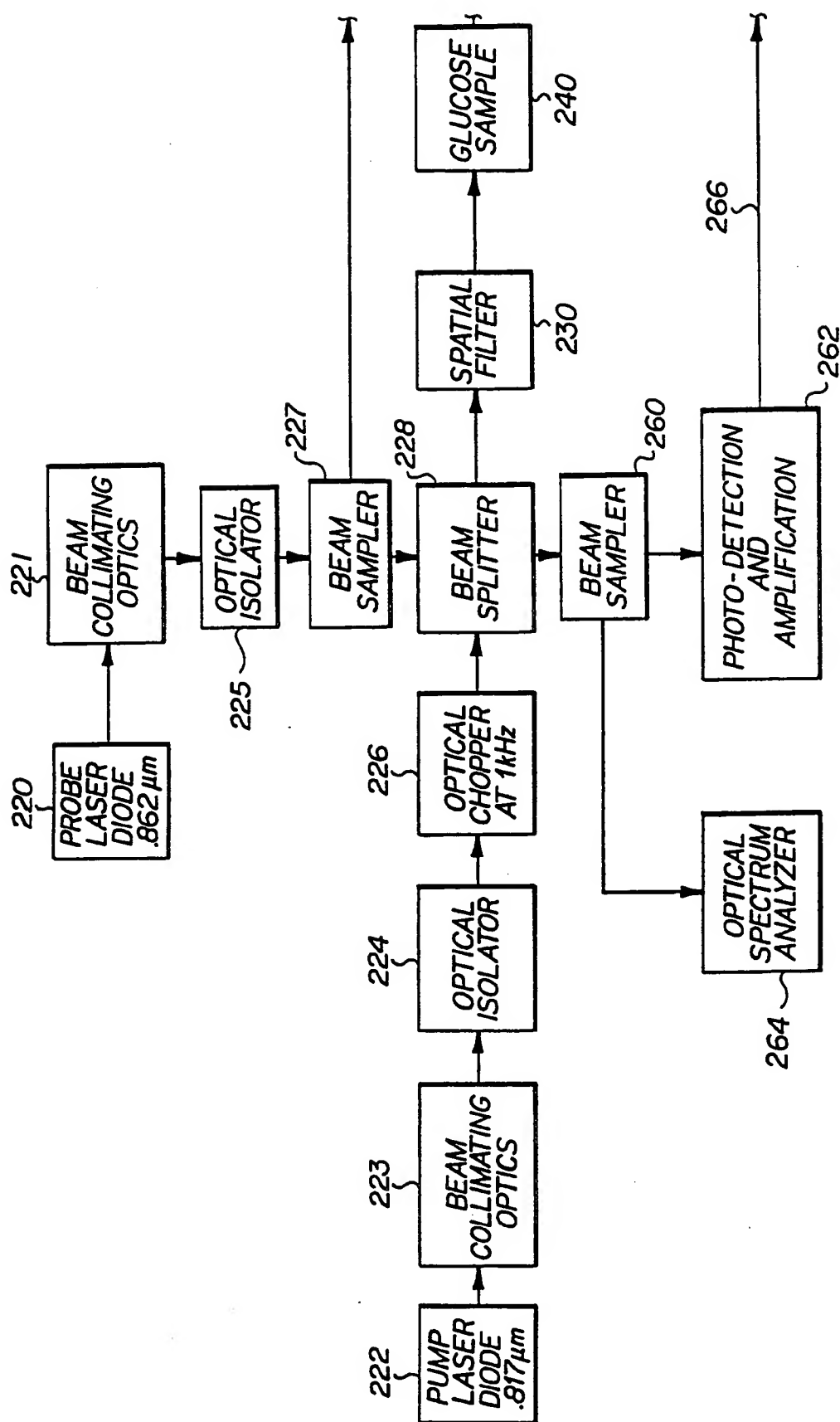
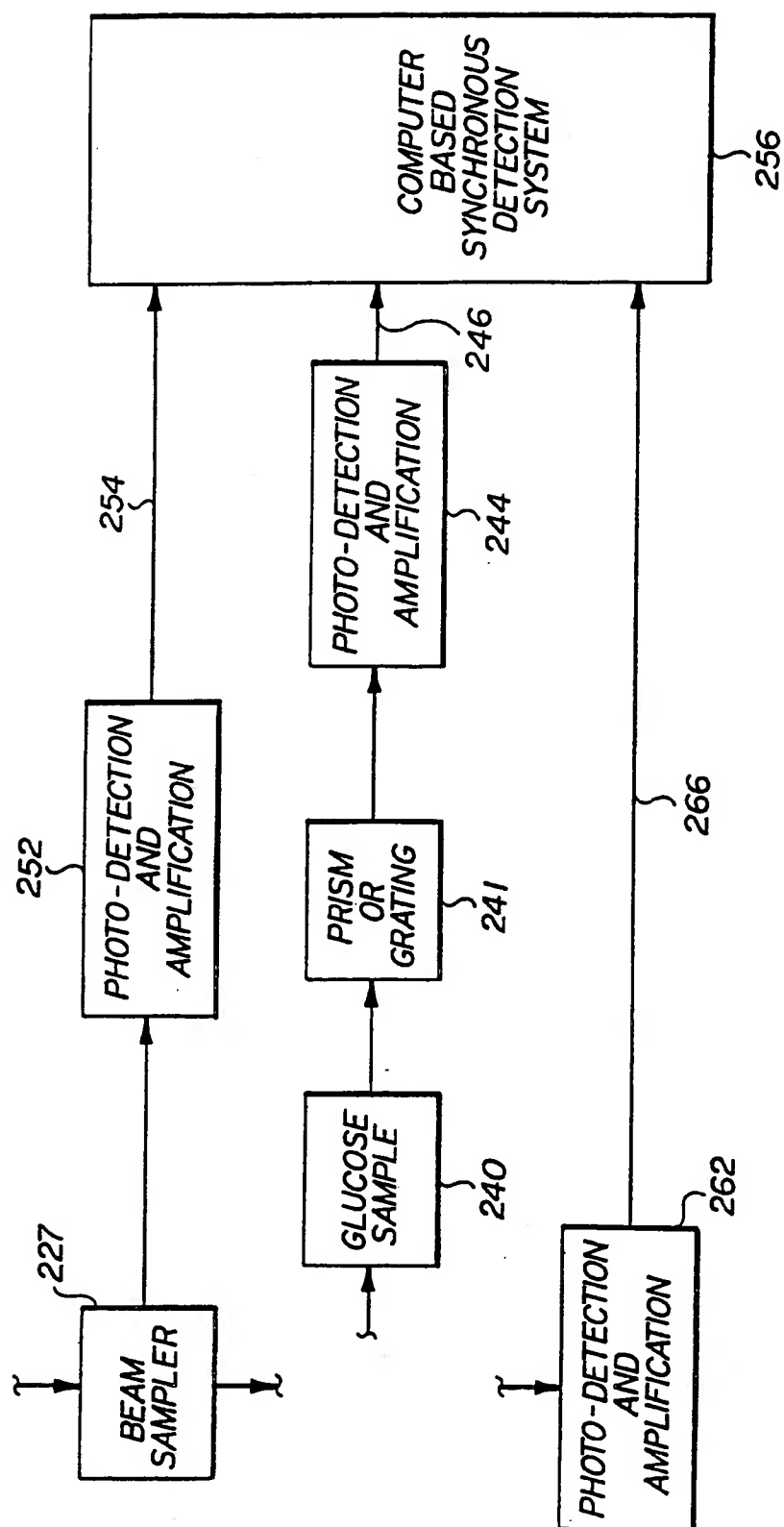


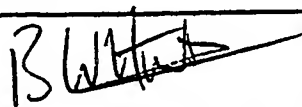
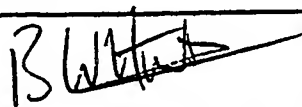
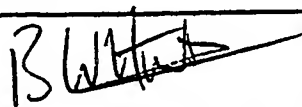
FIG 5

**FIG 6****FIG 7**

**FIG 8**

**FIG 9A**

**FIG 9B**

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A61B5/00											
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">Int.Cl. 5</td> <td style="padding: 5px;">A61B ; G01N</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched⁸</div>			Classification System	Classification Symbols	Int.Cl. 5	A61B ; G01N					
Classification System	Classification Symbols										
Int.Cl. 5	A61B ; G01N										
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category⁹</th> <th style="width: 70%; padding: 5px;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 20%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;"> DIABETES CARE vol. 5, no. 3, May 1982, pages 254 - 258; W.F.MARCH ET AL.: 'Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part I. Measurement of Very Small Optical Rotations' cited in the application see the whole document <div style="text-align: center;">---</div> </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1, 12, 27</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;"> DIABETES CARE vol. 5, no. 3, May 1982, pages 259 - 265; W.F.MARCH ET AL.: 'Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part II. Animal Studies and the Scleral Lens' cited in the application see the whole document <div style="text-align: center;">---</div> <div style="text-align: center;">-/-</div> </td> <td style="text-align: center; vertical-align: top; padding: 5px;">1, 12, 27</td> </tr> </tbody> </table>			Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	DIABETES CARE vol. 5, no. 3, May 1982, pages 254 - 258; W.F.MARCH ET AL.: 'Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part I. Measurement of Very Small Optical Rotations' cited in the application see the whole document <div style="text-align: center;">---</div>	1, 12, 27	A	DIABETES CARE vol. 5, no. 3, May 1982, pages 259 - 265; W.F.MARCH ET AL.: 'Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part II. Animal Studies and the Scleral Lens' cited in the application see the whole document <div style="text-align: center;">---</div> <div style="text-align: center;">-/-</div>	1, 12, 27
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>⁹ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>											
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center;">12 MARCH 1992</div> </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center;">24. 03. 92</div> </td> </tr> <tr> <td style="padding: 5px;"> International Searching Authority <div style="text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">HUNT B.W. </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center;">12 MARCH 1992</div>	Date of Mailing of this International Search Report <div style="text-align: center;">24. 03. 92</div>	International Searching Authority <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">HUNT B.W. </div>					
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^a	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	OPTICS LETTERS. vol. 1, no. 5, November 1977, NEW YORK US pages 152 - 154; A.OWYOUNG ET AL.: 'Stimulated Raman spectroscopy using low-power cw lasers' cited in the application see the whole document ---	1,12,27
A	US,A,4 832 483 (S.P.VERMA) 23 May 1989 see the whole document ---	1,12,27
A	US,A,4 014 321 (W.F.MARCH) 29 March 1977 see the whole document ---	1,12,27

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9108416
SA 54187**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4832483	23-05-89	None	
US-A-4014321	29-03-77	US-A- 3958560	25-05-76
		DE-A- 2538985	26-05-76
		GB-A- 1521113	09-08-78
		JP-A- 51075498	30-06-76

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(71) Applicant (for all designated States except US): MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only) : RAVA, Richard, P. [US/US]; 635 Beaver Street, Waltham, MA 02154 (US). BARAGA, Joseph, J. [US/US]; 109 Highland Avenue, Apt. 30, Somerville, MA 02143 (US). FELD, Michael, S. [US/US]; 56 Hinckley Road, Waban, MA 02168 (US).

(74) Agents: HOOVER, Thomas, O. et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.

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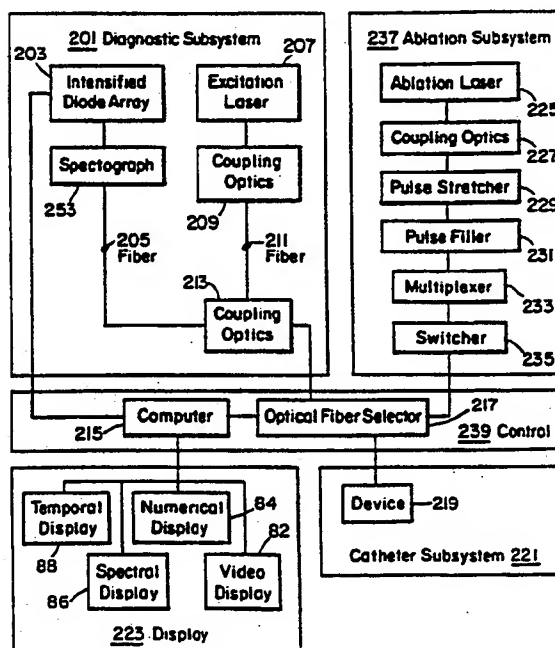
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: SYSTEMS AND METHODS OF MOLECULAR SPECTROSCOPY TO PROVIDE FOR THE DIAGNOSIS OF TISSUE

(57) Abstract

Systems and methods for spectroscopic diagnosis and treatment are employed which utilize molecular spectroscopy to accurately diagnose the condition of tissue. Infrared Raman spectroscopy and infrared attenuated total reflectance measurements are performed utilizing a laser radiation source and a Fourier transform spectrometer. Information acquired and analyzed in accordance with the invention provides accurate details of biochemical composition and pathologic condition.



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SYSTEMS AND METHODS OF MOLECULAR SPECTROSCOPY
TO PROVIDE FOR THE DIAGNOSIS OF TISSUE

Related to U.S. Application

This application is a continuation-in-part of
5 "Systems and Methods of Molecular Spectroscopy to
Provide for the Diagnosis of Tissue", U.S. Serial
No. 07/661,077 filed on February 26, 1991 by Richard
P. Rava, Joseph J. Baraga, and Michael S. Feld, and
is incorporated herein by reference. This
10 application is also related to "Devices and Methods
For Optical Diagnosis of Tissue" filed on February
26, 1991 by G. Sargent Janes and Gary B. Hayes which
corresponds to U.S. Serial No. 07/661,072 and is
incorporated herein by reference.

15 Government Support

Funding for research conducted in connection
with the subject matter of the present application
was provided under NIH Grant No. RR 02594.

Background of the Invention

20 In the United States heart attacks, almost
entirely attributable to coronary atherosclerosis,
account for 20-25% of all deaths. Several medical
and surgical therapies are available for treatment
of atherosclerosis; however, at present no in situ
25 methods exist to provide information in advance as
to which lesions will progress despite a particular
medical therapy.

Objective clinical assessments of
atherosclerotic vessels are at present furnished
30 almost exclusively by angiography, which provides

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anatomical information regarding plaque size and shape as well the degree of vessel stenosis. The decision of whether an interventional procedure is necessary and the choice of appropriate treatment modality is usually based on this information. However, the histological and biochemical composition of atherosclerotic plaques vary considerably, depending on the stage of the plaque and perhaps also reflecting the presence of multiple etiologies. This variation may influence both the prognosis of a given lesion as well as the success of a given treatment. Such data, if available, might significantly assist in the proper clinical management of atherosclerotic plaques, as well as in the development of a basic understanding of the pathogenesis of atherosclerosis.

At present biochemical and histological data regarding plaque composition can only be obtained either after treatment, by analyzing removed material, or at autopsy. Plaque biopsy is contraindicated due to the attendant risks involved in removing sufficient arterial tissue for laboratory analysis. Recognizing this limitation, a number of researchers have investigated optical spectroscopic methods as a means of assessing plaque deposits. Such "optical biopsies" are non-destructive, as they do not require removal of tissue, and can be performed rapidly with optical fibers and arterial catheters. With these methods, the clinician can obtain, with little additional risk to the patient, information that is necessary to predict which lesions may progress and to select the best treatment for a given lesion.

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Among optical methods, most attention has centered on ultraviolet and/or visible fluorescence. Fluorescence spectroscopy has been utilized to diagnose disease in a number of human tissues, including arterial wall. In arterial wall, fluorescence of the tissue has provided for the characterization of normal and atherosclerotic artery. However the information provided is limited by the broad line width of fluorescence emission signals. Furthermore, for the most part, fluorescence based methods provide information about the electronic structure of the constituent molecules of the sample. There is a need for non-destructive real time biopsy methods which provide more complete and accurate biochemical and molecular diagnostic information. This is true for atherosclerosis as well as other diseases which affect the other organs of the body.

Summary of the Invention

The present invention relates to vibrational spectroscopic methods using Fourier transform infrared (FT-IR) attenuated total reflectance (ATR) and near-infrared (IR) FT-Raman spectroscopy. These methods provide extensive molecular level information about the pathogenesis of disease. Both of these vibrational techniques are readily carried out remotely using fiber optic probes. In particular, a preferred embodiment utilizes FT-Raman spectra of human artery for distinguishing normal and atherosclerotic tissue. Near IR FT-Raman spectroscopy can provide information about the tissue state which is unavailable from fluorescence

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methods. In situ vibrational spectroscopic techniques allow probing of the molecular level changes taking place during disease progression. The information provided is used to guide the choice of the correct treatment modality.

These methods include the steps of irradiating the tissue to be diagnosed with radiation in the infrared range of the electromagnetic spectrum, detecting light emitted by the tissue at the same frequency, or alternatively, within a range of frequencies on one or both sides of the irradiating light, and analyzing the detected light to diagnose its condition. Both the Raman and ATR methods are based on the acquisition of information about molecular vibrations which occur in the range of wavelengths between 3 and 300 microns. Note that with respect to the use of Raman shifted light, excitation wavelengths in the ultraviolet, visible and infrared ranges can all produce diagnostically useful information. Near IR FT-Raman spectroscopy is ideally suited to the study of human tissue.

Raman spectroscopy is an important method in the study of biological samples, in general because of the ability of this method to obtain vibrational spectroscopic information from any sample state (gas, liquid or solid) and the weak interference from the water Raman signal in the "fingerprint" spectral region. The FT-spectrometer furnishes high throughput and wavelength accuracy which might be needed to obtain signals from tissue and measure small frequency shifts that are taking place. Finally, standard quartz optical fibers can be used to excite and collect signals remotely.

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Near IR FT-Raman spectroscopy provides the capability to probe biological substituents many hundred microns below the tissue surface. In particular, for atherosclerotic tissue, calcified deposits below the tissue surface are easily discerned. Thus, it becomes possible to detect pathologic conditions which would not be apparent using angioscopic methods, as well as to study the detailed molecular basis of the pathology.

In contrast with electronic techniques, the bands in a vibrational spectrum are relatively narrow and easy to resolve. Vibrational bands are readily assigned to individual molecular groups.

The ATR technique offers several features especially suited to sampling of human tissue in vivo. Being a surface technique, the ATR method can non-destructively probe internal human tissue either by direct contact in a hollow organ (e.g. artery), or by insertion of a needle probe. In the mid-IR region, strong water absorption dominates the spectra of highly hydrated samples such as arterial tissue, obscuring the absorption from other tissue components (see Figure 8). Accurate subtraction of the strong water absorption from FT-IR ATR spectra is relatively easy and very reliable with the high dynamic range, linearity, stability, and wavelength precision of available FT spectrometers. Furthermore, high quality mid-IR spectra of aqueous protein solutions can be collected with fiber optic ATR probes. Such probes are easily adaptable to existing catheters for remote, non-destructive measurements in vivo. The mid-IR ATR technique allows clinicians to gather precise histological and

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biochemical data from a variety of tissues during standard catheterization procedures with minimal additional risk.

5 The present methods relate to infrared methods of spectroscopy of various types of tissue and disease including cancerous and pre-cancerous tissue, non-malignant tumors or lesions and atherosclerotic human artery. Examples of measurements on human artery generally illustrate
10 the utility of these spectroscopic techniques for clinical pathology. Results obtained demonstrate that high quality, reproducible FT-IR ATR spectra of human artery can be obtained with relative ease and speed. In addition, molecular level details can be
15 reliably deduced from the spectra, and this information can be used to determine the biochemical composition of various tissues including the concentration of molecular constituents that have been precisely correlated with disease states to
20 provide accurate diagnosis.

Another preferred embodiment of the present invention uses two or more diagnostic procedures either simultaneously or sequentially collected to provide for a more complete diagnosis. These
25 methods can include the use of fluorescence of endogenous tissue, Raman shifted measurements and/or ATR measurements.

Yet another preferred embodiment of the present invention features a single stage spectrograph and
30 charge-coupled device (CCD) detector to collect NIR Raman spectra of the human artery. One particular embodiment employs laser light in the 810 nm range to illuminate the tissue and thereby provide Raman

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spectra having frequency components in a range suitable for detection by the CCD. Other wavelengths can be employed to optimize the diagnostic information depending upon the particular type of tissue and the type and stage of disease or abnormality. Raman spectra can be collected by the CCD at two slightly different illumination frequencies and are subtracted from one another to remove broadband fluorescence light components and thereby produce a high quality Raman spectrum. The high sensitivity of the CCD detector combined with the spectra subtraction technique allow high quality Raman spectra to be produced in less than 1 second with laser illumination intensity similar to that for the FT-Raman system also described herein.

Brief Description of the Drawings

Figures 1A-1C are schematic illustrations of preferred systems for providing the spectroscopic measurements of the invention.

Figure 2 graphically illustrates FT-Raman spectra of human aorta: a) normal artery; b) atheromatous plaque; c) FT-Raman spectrum solid cholesterol (Sigma).

Figure 3 graphically illustrates FT-Raman spectra of normal human aorta: a) irradiated from intimal side (spectrum multiplied by 3); and b) irradiated from adventitial side (primary adipose tissue). c) NIR FT-Raman spectrum of triglyceride, triolein.

Figure 4 graphically illustrates FT-Raman spectra from human aorta: a) fibrous plaque; and

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b) atheromatous plaque. c) FT-Raman spectrum of cholesterol monohydrate powder.

Figure 5 graphically illustrates FT-Raman spectra of calcified human aorta: a) calcified with fibrous cap; b) excised calcification from a different plaque; c) spectra of the same tissue as in a) taken from adventitial side.

Figure 6 graphically illustrates FT-Raman spectra of calcified human aorta: a) calcified plaque with a fibrous cap (spectrum multiplied by 8); and b) exposed calcification.

Figure 7 graphically illustrates the measured NIR Raman intensity of the 960 cm^{-1} band ($A(960\text{ cm}^{-1})$ indicates the area of this band) in a calcified deposit as a function of depth below the irradiated surface. The dashed curve corresponds to the fit of an exponential function to the data with an exponent of 2.94 mm^{-1} .

Figure 8 graphically illustrates FT-IR ATR spectra ($4000 - 700\text{ cm}^{-1}$) of (a) normal aorta, intimal surface; and (b) buffered saline (0.14M NaCl , pH 7.4).

Figure 9 graphically illustrates FT-IR ATR spectra ($1800 - 800\text{ cm}^{-1}$) after water subtraction: (a) Normal aorta, intimal surface; (b) Sub-adventitial fat; (c) Saline rinsed from the intimal surface of normal aorta.

Figure 10 graphically illustrates FT-IR ATR spectra ($1800 - 800\text{ cm}^{-1}$): (a) Two consecutive water-subtracted spectra of normal aorta, intimal surface, collected immediately after placement on ATR element (solid line) and 10 minutes later

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(dashed line); (b) Same two spectra as in (a) after lipid subtraction, scaled to have equal maxima.

Figure 11 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}), water-and lipid-subtracted: (a) Normal aorta, media layer; (b) Atherosclerotic plaque, intimal surface; (c) Atheromatous plaque with intact fibrous cap, intimal surface.

Figure 12 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}): (a) Necrotic core of atheromatous plaque, water-and lipid-subtracted; (b) Dry film of cholesterol.

Figure 13 graphically illustrates scatter plot for all samples of the area, A(1050), of the 1050 cm^{-1} cholesterol band (integrated from 1075 to 1000 cm^{-1}) ratioed to the area, A(1550) of the 1548 cm^{-1} protein amide II band (integrated from 1593 to 1485 cm^{-1}). The intensities were calculated from the water-and lipid-subtracted spectra. NORMAL denotes normal aorta specimens, intimal side, FIBROUS includes atherosclerotic and atheromatous plaques with intact fibrous caps, and NECROTIC includes exposed necrotic atheroma cores and necrotic material isolated from atheromatous plaques.

Figure 14 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}): (a) Second derivative spectrum of normal aorta intima (Figure 8a); (b) Water-subtracted spectrum of same normal aorta intima specimen (same as Figure 9a).

Figure 15 graphically illustrates a scatter diagram for all the specimens of the area, A(1050)

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(dashed line); (b) Same two spectra as in (a) after lipid subtraction, scaled to have equal maxima.

Figure 11 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}), water-and lipid-subtracted: (a) Normal aorta, media layer; (b) Atherosclerotic plaque, intimal surface; (c) Atheromatous plaque with intact fibrous cap, intimal surface.

Figure 12 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}): (a) Necrotic core of atheromatous plaque, water-and lipid-subtracted; (b) Dry film of cholesterol.

Figure 13 graphically illustrates scatter plot for all samples of the area, $A(1050)$, of the 1050 cm^{-1} cholesterol band (integrated from 1075 to 1000 cm^{-1}) ratioed to the area, $A(1550)$ of the 1548 cm^{-1} protein amide II band (integrated from 1593 to 1485 cm^{-1}). The intensities were calculated from the water-and lipid-subtracted spectra. NORMAL denotes normal aorta specimens, intimal side, FIBROUS includes atherosclerotic and atheromatous plaques with intact fibrous caps, and NECROTIC includes exposed necrotic atheroma cores and necrotic material isolated from atheromatous plaques.

Figure 14 graphically illustrates FT-IR ATR spectra (1800 - 800 cm^{-1}): (a) Second derivative spectrum of normal aorta intima (Figure 8a); (b) Water-subtracted spectrum of same normal aorta intima specimen (same as Figure 9a).

Figure 15 graphically illustrates a scatter diagram for all the specimens of the area, $A(1050)$

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of the 1050 cm^{-1} cholesterol band plotted versus the area, $A(1382)$, of the 1382 cm^{-1} cholesterol band. Both cholesterol bands have been normalized to the area, $A(1050)$, of the protein amide II band. All
5 band intensities were calculated from the water-and lipid-subtracted spectra. Tissue categories are the same as in Figure 13. The solid line represents a linear least squares fit to the data.

Figures 16A and 16B are additional preferred
10 embodiments of ATR probes adapted to make the diagnostic measurements of the present invention.

Figure 17 is a schematic diagram of the system of Figure 1A modified to use the spectrograph/CCD Raman detector of the present invention.

15 Figure 18 is a schematic diagram of a preferred system for implementing the spectrograph/CCD Raman detector of the present invention.

Figure 19 graphically illustrates spectrograph/CCD-Raman spectra of normal human
20 aorta: A) Raman plus fluorescence spectrum produced by illuminating the tissue sample with 810 nm laser light; B) Raman difference spectrum produced by subtracting spectra produced by illuminating the tissue sample with 810 and 812 nm laser light; C)
25 resulting Raman spectrum produced by integrating the Raman difference spectrum of B).

Figure 20 graphically illustrates spectrograph/CCD-Raman spectra of an atherosclerotic plaque with a calcified deposit exposed at the
30 surface: A) Raman plus fluorescence spectrum produced by illuminating the tissue sample with 810 nm laser light; B) Raman difference spectrum

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produced by subtracting spectra produced by illuminating the tissue sample with 810 and 812 nm laser light; C) resulting Raman spectrum produced by integrating the Raman difference spectrum of B).

5 Figure 21 graphically illustrates a spectrograph/CCD-Raman spectrum of adventitial adipose tissue.

Detailed Description

10 The spectroscopic methods of the present invention can be performed on a system such as that for laser treatment of atherosclerosis which is illustrated in Figure 1A. Figure 1A includes separate block diagrams for the system of the invention which utilizes laser light for
15 spectroscopic diagnosis as well as for treatment and/or removal of tissue. The ablation laser 225, pulse stretcher 229 and the pulse filler/multiplexer 231, 233 produce an output laser ablation pulse of sufficient energy and intensity to remove tissue and
20 sufficient pulse duration to propagate through a fiber optic laser catheter delivery system without damaging the fibers. These systems and methods are more fully described in co-pending application U.S. Serial No. 07/644,202 filed on January 22, 1991,
25 which is incorporated herein by reference.

 To remove plaque, a device 219 is used to contact the tissue such as multiple-fiber laser catheter 10 of Figure 1B with an optical shield. The catheter 10 is inserted into the artery and the
30 distal end of the catheter is brought into contact with the lesion. Next, a determination is made as to the type of tissue at which each optical fiber

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20a-c' is aimed. Only fibers aimed at diseased tissue are activated. Thus, selective tissue removal is obtained. Furthermore, this technique is also applicable for guiding surgical procedures in
5 other organs and tissue types such as the colon and bladder.

The present invention relates to systems and methods of performing spectral diagnostics to diagnose the tissue in front of each fiber. A
10 preferred embodiment a laser light source 207 that is coupled to the fibers. The diagnostic light is sent to the fiber of choice by the optical fiber selector 217.

The diagnostic light exits the selected optical
15 fiber and falls on the tissue. The tissue absorbs the light and a fraction of the absorbed light is re-emitted, by Rayleigh fluorescence, Raman or other elastic or inelastic light scattering processes. This light is returned to the optical fibers and
20 exits from selector 217, and is detected by a photodiode, photomultiplier or other detector 203. Returning light could use different optical fibers than those employed for illumination. Diagnostic subsystem produces the entire spectral signal which
25 is coupled to computer 80.

The computer stores the information in a memory as a spectrum, which is a graph of light intensity vs. wavelength. This can be displayed immediately on the video display 82 or compared to an existing
30 spectrum stored in the computer and the difference displayed on the spectral display 86. Temporal display 88 can display corrections made for the wavelength dependent sensitivities of the source.

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Information from either the temporal or spectral display can be stored in the computer 80. The comparative data is shown on numerical display 84 to provide a quantitative measure of the health of the tissue observed.

With a multichannel detector and a computer, or with appropriate multiple filters and detectors, it is possible to gather this information in a fraction of a second. Thus, a spectral or numerical display is provided which indicates the type of tissue at which the fiber of interest is aimed. If the tissue is plaque, and is to be removed, then fiber selector 217 will align this fiber with the output beam of the high power laser 225. Then, the high power laser 225 is turned on and an appropriate power level is selected for a predetermined amount of time to remove a certain amount of diseased tissue. The beam of laser 225 is transmitted to pulse stretcher 229 and pulse filler/multiplexer 231, 233 to properly adjust the beam fluence.

The procedure is repeated for different fibers. Where diseased tissue is detected, it is quickly removed. The laser catheter 10 nibbles away at the plaque, leaving the healthy artery wall intact.

If the artery 30 makes a bend 31 as shown by Figure 1B, the laser catheter 10 will tend to make contact with artery wall 32 at the outside wall of the bend. To prevent the catheter from contacting the artery wall, the optical fiber 20c is not fired. The lesion is removed asymmetrically. This allows the laser catheter 10 to follow the lumen 39, 39a around the bend. Thus, the artery wall 32 is not irradiated and is not perforated. Additional

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details of this fiber optic catheter 10 are disclosed in U.S. Patent No. 4,913,142, the contents of which are incorporated herein by reference.

5 In both Raman and ATR methods, information is contained in the spectral lines which are observed in their intensities, and also their linewidths and center frequencies (and how they change in different environments). Further, Raman and infrared ATR have different "selection rules". Some vibrations seen
10 in infrared won't show up in Raman, and vice versa. In other cases the same vibration can be detected by both techniques, but with different relative intensities (e.g. a strong Raman line will be weak in ATR). So in this sense the two techniques
15 provide complementary information and combining the two techniques (or using either or both with laser induced fluorescence) is valuable in diagnosing pathology.

The methods can utilize Fourier transform
20 detection to observe the radiation thereby providing improved signal/noise ratios. Other techniques (e.g. diode array detection and CCD detection) can also be used.

As described in more detail below contributions
25 from major tissue constituents can be "subtracted out" to reveal information about molecules which are present in small concentrations. For example, in ATR water contributions are removed before the "dry" tissue constituents could be studied. Also,
30 derivative spectroscopy is used to eliminate background signals and low frequency filters. Note that these techniques deconvolute the observed spectra into its individual constituents, an

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essential step for optimal extraction of diagnostic information.

While Raman can sample deeply into tissue, ATR samples only a very thin layer (a few microns).

5 Thus, ATR is "naturally" suited to probe surface disease, such as the superficial cancers of the bladder and GI tract, whereas Raman is well suited to providing information about conditions deep inside tissue (such as breast cancer or stones).

10 This is important for 3D imaging. Furthermore, the ATR tissue sampling depth can be controlled by properly matching the probe surface material to the tissue type.

Generally, the ATR signal is very sensitive to
15 the surface of the waveguide or probe. For example, if the probe surface has an affinity for lipids in the tissue, lipids can migrate to the probe surface, creating a thin lipid layer and producing a large signal. This can be a problem (it can give
20 misleading information if not properly recognized and guarded against). Conversely, it can be used to advantage: Probes with special surfaces can be developed to prevent this effect or to promote it, in order to search for particular substances in the
25 tissue.

In a preferred method one can adjust depth probed by ATR by varying refractive index of ATR probe. Alternatively as discussed below one can use a "waveguide needle" to get subsurface information.

30 Raman diagnostic methods permit adjustment of Raman depth by varying the wavelength. Penetration depth is wavelength dependent, and can be varied by choosing different excitation wavelengths between

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about $\lambda=700\text{nm}$ and $2\mu\text{m}$. Another potentially important way of adjusting Raman depth is to vary the collection angle. In the near IR, incident (exciting) light is strongly scattered out of the forward direction into larger angles, so Raman signals sampled at smaller angles come from tissue closer to the surface. Therefore, the Raman sampling depth can be controlled to a large extent by probe design.

Depth information is important if one desires to provide imaging by creating 3D images of small tumors in the brain or breast. Differential techniques based on the ideas of the preceding paragraph might allow accurate localization of such tumors in three dimensions. Near-IR Raman can be combined with a sound wave technique (time of flight or standing waves set up in the tissue)--the sound wave would modulate the Raman signal emanating from a point in the tissue when it passes that point, and the modulated signal could be used to establish the depth of the tissue producing the signal.

A system employed for the collection of Raman spectral data from excised tissue samples is illustrated in Figure 1C. FT-Raman spectra were measured from 0 - 4000cm^{-1} below the laser excitation frequency with a FT-IR interferometer 40 equipped with a FT-Raman accessory. The accessory employed at 180 back scattering geometry and a cooled (77K) InGaAs detector 42.

A 1064 nm CW Nd:YAG laser 44 can be used for irradiating a sample 46: utilizing 500 mW to 1 W laser power in a 1.0 to 2.5 mm spot 48 at the sample

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46 to collect Raman data. Alternatively, a pulsed laser source can also be employed. Laser 44 generated a beam 46 that is directed through plasma filter 48, mirrors 50, 52, focussing lens 54 and mirror or prism 56 before irradiating the sample 46. The radiation received by sample 46 undergoes various mechanisms of absorption, reflection and scattering including Raman scattering. Some of the light emitted by the tissue is directed toward lens 60 and then through one or more Rayleigh filters 62. The collecting lens 60 collects this backscattered light 64 and collimates it. The Rayleigh filters 62 removes the elastically scattered light and transmits the inelastically scattered, frequency shifted (Raman) light. The Raman scattered light enters the interferometer 40. No visible sample degradation was observed under these conditions.

Excised human aorta was chosen of atherosclerotic artery tissue. Samples were obtained at the time of post mortem examination, rinsed with isotonic saline solution (buffered at pH 7.4), snap-frozen in liquid nitrogen, and stored at -85C until use. Prior to spectroscopic study, samples were passively warmed to room temperature and were kept moist with the isotonic saline. Normal and atherosclerotic areas of tissue were identified by gross inspection, separated, and sliced into roughly 8x8 mm pieces.

The tissue samples were placed in a suprasil quartz cuvette with a small amount of isotonic saline to keep the tissue moist, with one surface in contact with the irradiated by the laser 44. The spectra shown in Figures 2 through 6 were collected

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with 512 scans at 8 cm^{-1} resolution (approximately 35 minutes total collection time).

Human aorta is composed of three distinct layers: intima, media, and adventitia. The intima, normally less than $300\text{ }\mu\text{m}$ thick, is the innermost layer and provides a non-thrombogenic surface for blood flow. It is mainly composed of collagen fibers and ground substance. The medial layer, typically about $500\text{ }\mu\text{m}$ thick, is quite elastic and serves to smooth the pulsatile blood flow from the heart. The structural protein elastin is the major component of aortic media, with some smooth muscle cells present as well. The outermost adventitial layer serves as a connective tissue network which loosely anchors the vessel in place, and is mainly made up of lipids, lipoproteins and collagen. During the atherosclerotic process, the intima thickens due to collagen proliferation, fatty necrotic deposits accumulate under and within the collagenous intima, and eventually, calcium builds up, leading to calcium hydroxyapatite deposits in the artery wall.

Figure 2a shows the FT-Raman spectrum of a full thickness section of aorta grossly identified as normal. Laser irradiation was on the intimal side. The dominant bands appear at 1669 cm^{-1} and 1452 cm^{-1} and can be assigned to an amide I backbone and C-H in-plane bending vibration from proteins, respectively. Weaker bands at 1331 and 1255 cm^{-1} are assigned to C-H wagging and amide III vibrations from proteins, respectively. The frequencies of

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amide I and III are consistent with those observed for disordered proteins.

Another example of a typical NIR FT Raman spectrum from normal aorta is shown in Figure 3. When irradiated from the intimal side, Figure 3a, the major vibrational bands observed in normal aorta are all attributable to protein vibrations: the band at 1658 cm^{-1} is assigned to the amide I vibration of the polypeptide chain, the 1453 cm^{-1} band to a C-H bending mode of proteins, and the 1252 cm^{-1} band to the amide III vibration. The spectrum of normal aorta is at least 25% weaker than any of the pathologic samples. The peak frequency of the C-H bending band, which averaged for all the normal specimens is $1451 \pm 1 \text{ cm}^{-1}$, is specific to the protein C-H bending mode (See below). The weak band near 1335 cm^{-1} , which appears as a shoulder in many of the normal specimens, appears to be specific to elastin, and the weak band at 1004 cm^{-1} is likely due to phenylalanine residues. In general, the relative intensities of the bands in the region between 1250 and 1340 cm^{-1} appears very much like that observed in the FT Raman spectrum of elastin. This observation is consistent with the thin collagenous intima in normal aorta, the elastic nature of the media of aorta, and the deep penetration depth of 1064 nm radiation. Band assignments for all tissue types presented here are listed in Table 2.

Figure 3b displays the NIR FT Raman spectrum of the adventitial side of normal aorta. In this case, the irradiated adventitial surface consisted of several millimeters of visible adipose tissue. In

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contrast with the spectrum collected from the intimal side, the bands observed in this adipose material appear to be mainly due to lipid, and in particular triglyceride, with almost no contribution from protein. This is not unexpected, as the triglyceride content of adipose tissue is on the order of 60%. The sharp band at 1655 cm^{-1} is due to stretching of C=C groups in unsaturated fatty acid chains. This band is distinguished from amide I by its peak frequency and its width, which in this case is 17 cm^{-1} FWHM. Amide I, in contrast, is roughly 60 cm^{-1} wide. The dominant C-H bending band is shifted to 1440 cm^{-1} , characteristic of lipids. This band is about 3 times more intense in adipose tissue than in normal intima, probably a result of the greater number of C-H groups per unit volume in triglycerides. The bands at $1301/1267\text{ cm}^{-1}$ and 1080 cm^{-1} are assigned to C-H bending and C-C stretching vibrations of fatty acids, respectively.

The 1746 cm^{-1} band, assigned to the C=O stretching mode of the triglyceride ester linkages, indicates that most of the lipid observed in the adventitial adipose tissue is of the triglyceride form. Specifically, the integrated intensity of this band relative to the C-H bending band at 1440 cm^{-1} is equal to 0.103, while this same ratio for triolein is 0.136, which indicates that roughly 75% of the C-H band is due to triglyceride. The NIR FT Raman spectrum of triolein (a triglyceride containing fatty acid chains of 18 carbons and a single double bond) is shown for comparison in Figure 3c. Additional molecular information

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regarding the state of the fatty acid chains is readily deduced from the adventitial adipose spectrum. For example, the relative intensity of the C=C band at 1655 cm^{-1} indicates that there are on average roughly 0.7 unsaturated double bonds per fatty acid chain, assuming 16-18 carbon fatty acids. In addition, the frequencies and structures of the C-H bending and C-C stretching bands suggest that most of the fatty acid chains are in the *gauche* conformation. The sharp 1129 cm^{-1} band, characteristic of all-trans chains, is not observed in the spectrum.

The FT-Raman spectrum obtained from a diseased artery, an atheromatous plaque, with a thick fibrous connective tissue cap and an underlying necrotic core is shown in Figure 2b. The necrotic core of an atheromatous plaque contains cellular debris as well as large accumulations of oxidized lipids and cholesterol. The band in the amide I region, peaking at 1665 cm^{-1} , is distinctly narrower in this spectrum compared to normal aorta. In addition, the in-plane C-H bend, at 1444 cm^{-1} , is relatively more intense and has a distinct shoulder at higher frequency. The two weaker bands at 1307 and 1267 cm^{-1} are shifted in frequency from those found in the spectrum of normal aorta. The band frequencies and shapes in the FT-Raman spectrum of cholesterol, shown in Figure 2c, coincide with some of those observed in the atheromatous plaque, consistent with the expected composition of the necrotic core.

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The NIR FT Raman spectra of other fibrous plaque specimens exhibit a range of features as shown in Figures 4 and 5. Figure 4a shows a representative spectrum from one of the types of fibrous plaques. These fibrous plaque spectra are quite similar in both relative and absolute band intensities to the spectra of normal aorta. The most significant differences are that the C-H bending band, peaking near 1448 cm^{-1} on average, is shifted by 2 cm^{-1} to a slightly lower frequency. This may be the result of a small increase in the lipid content of these plaques relative to normal aorta. In addition, the band near 1340 cm^{-1} , attributed to elastin in normal aorta, is decreased relative to amide III at 1265 cm^{-1} . The putative explanation is that the collagenous intima is thickened in these specimens, so that the spectral contribution from the elastic media is reduced relative to that of normal aorta.

The NIR FT Raman spectra of other fibrous plaque specimens appeared similar to atheromatous plaques' spectra (Figure 2b). These are substantially different than either normal aorta, or adipose tissue. In these cases, the intense C-H bending band occurs at 1440 cm^{-1} , characteristic of lipid material. This band is roughly twice as intense as the C-H bending band in normal aorta. The complete absence of a band at 1746 cm^{-1} indicates that this lipid is not triglyceride. In fact, this lipid appears to be predominantly cholesterol, as identified by the sharp, characteristic band at 700 cm^{-1} and comparison to the cholesterol spectrum shown

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in Figure 4c. Again, this is not surprising, since
cholesterols accumulate in high concentrations in
atherosclerotic lesions. Several of the bands
between 1000 and 500 cm^{-1} are assignable to
5 vibrational modes of the sterol rings. These
include the bands at 959, 882, 844, 805, 700, 605,
and 546 cm^{-1} . In addition, the 1666 cm^{-1} band is
attributed in part to the C=C stretching vibration
of the steroid nucleus.

10 The presence of fatty acid chains in the
atheromatous plaque spectra is evidenced by bands at
1300/1262 cm^{-1} and 1130/1088 cm^{-1} , due to C-H bending
and C-C stretching vibrations, respectively. These
bands may contain contributions from cholesterol as
15 well. The relative intensities of the fatty acid
band at 1300 cm^{-1} and the sterol ring bands suggest a
mixture of free cholesterol and cholesterol-fatty
acid esters. Moreover, the relative intensities of
the 1130 cm^{-1} C-C stretching and the 700 cm^{-1} sterol
20 bands indicate that most of the fatty acid chains
are in the *gauche* conformation, consistent with the
predominance of unsaturated fatty acid chains in the
cholesterol esters in these plaques. It is
particularly noteworthy in the atheromatous plaques
25 that the cholesterol deposits are detected from
material below a thick fibrous cap indicating the
ability of the NIR FT Raman method to probe
materials several hundred microns below the tissue
surface.

30 In addition to the cholesterol and cholesterol
ester bands, the NIR FT Raman spectra of some of the
fibrous plaques contained two unique bands, at 1519

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and 1157 cm^{-1} . The intensities of these bands are highly correlated, which suggests that they are due to a single component. These bands, which have been previously observed in visibly-excited Raman spectra of atherosclerotic plaques, are assigned to carotenoids. The amount of carotenoid in these plaques is probably much smaller than the amounts of cholesterol or proteins, but may be strongly pre-resonance enhanced (14). The carotenoid bands are observed only in this subset of fibrous plaques.

In an advanced plaque, calcium may begin to accumulate leading to the formation of calcium hydroxyapatite crystals in the tissue as shown by the spectra of Figures 5 and 6. The FT-Raman spectrum of a calcified plaque with a thick (several hundred microns) fibrous connective tissue cap overlying a calcified deposit is shown in Figure 5a. The spectrum clearly indicates bands due to the protein in the fibrous cap, amide I and III at 1665 and 1255 cm^{-1} , respectively. However, additional bands are observed between 1250 and 1350 cm^{-1} and around 1100 cm^{-1} , as well as a strikingly sharp band at 961 cm^{-1} . The latter is readily assigned to the symmetric phosphate stretching vibration associated with phosphate groups in the calcium hydroxyapatite deposits, while the band around 1100 cm^{-1} is an asymmetric phosphate stretch. These assignments are confirmed by excising the solid "rock" from a different calcified plaque, and obtaining its spectrum as shown in Figure 5b. A strong Raman signal from the phosphate stretching vibration in solid calcifications in advanced atherosclerotic

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plaques can also be observed utilizing standard visible Raman instrumentation. The ability to detect the calcifications several hundred microns below the tissue surface when using near IR FT-Raman spectroscopy, however, is a diagnostic measurement which can be utilized to guide treatment.

A measurement was attempted to determine if the calcification might be detected when the tissue was irradiated from the adventitial side. The resulting FT-Raman spectrum is shown in Figure 5c. No evidence of the strong phosphate vibration is apparent. In contrast, sharp vibrational bands at 1745, 1656, 1444, 1303, 1267 and 1082 cm^{-1} are observed which are mainly associated with the lipid material that constitutes the majority of the adventitia.

The NIR FT Raman spectrum of calcified plaque, containing a subsurface calcified deposit and an overlying soft fibrous cap, exhibits an intense, sharp, new band at 960 cm^{-1} (Figure 6a). This band, specific to calcified tissue, is assigned to the symmetric stretching vibration of phosphate groups (15), which are present in high concentrations in the solid calcium salts. The weaker phosphate antisymmetric stretch is also present at 1072 cm^{-1} . A symmetric stretching vibration of carbonate groups may also contribute to this latter band. The phosphate vibrations are easily observed from subsurface deposits in the calcified plaques: the 960 cm^{-1} band can be observed from deposits up to 1.5 mm beneath a soft tissue cap with the current signal-to-noise level (See below). The calcified

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plaque also displays protein vibrations from the fibrous tissue cap. These include amide I at 1664 cm^{-1} and amide III near 1257 cm^{-1} . The C-H bending band at 1447 cm^{-1} suggests a mixture of protein and lipid, and the weak band at 699 cm^{-1} is likely due to cholesterol that is either in the fibrous cap, the calcified deposit, or both.

The NIR FT Raman spectra of exposed calcifications (Figure 6b) display a range of features. In all cases, the major bands are due to the calcium salts. These include the 960 cm^{-1} phosphate and 1072 cm^{-1} phosphate/carbonate bands as well as the band at 587 cm^{-1} , which is assigned to another phosphate vibrational mode. On the other hand, several differences are apparent in the weaker bands, which are presumably due to soft tissue components which are embedded in the calcification. In some cases (not shown), the C-H bending band occurs at 1450 cm^{-1} , and the band at 1663 cm^{-1} is similar in shape to amide I for some of the calcifications, indicative of protein vibrational modes. In other calcified plaques, such as that in Figure 5b, the C-H bending band occurs at 1440 cm^{-1} , and the 1667 cm^{-1} band, which is much sharper, is more like due to C=C stretching vibrations. In this plaque, the bands are due to lipid, in particular cholesterols, as evidenced by the 700 cm^{-1} and 1300 cm^{-1} bands.

In our histological examinations of aorta, two distinct types of exposed calcifications have been noted. In one type, the fibrous tissue cap is calcified. In the other, the necrotic core of an

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atheromatous plaque is calcified, and the calcified deposit is exposed by ulceration of the soft tissue fibrous cap. A positive explanation for the two spectral types of exposed calcifications is that the
5 specimens which exhibit protein bands are of the former histologic type, while the specimens which exhibit lipid bands are of the latter type.

The present methods provide an IR FT-Raman technique for differentiating various stages of
10 atherosclerosis in human aorta. They demonstrate that molecular level information is available using these methods. This information is useful for following the pathogenesis of the disease and in guiding the treatment of different lesions. The
15 near IR FT-Raman method, with its relatively deep penetration depth, is able to obtain spectroscopic signals from below the tissue surface, yielding details about the atheromatous necrotic tissue and sub-surface calcifications. These signals can be
20 utilized with an optical fiber based imaging system to determine the content and composition of different atherosclerotic plaques in vivo.

With the observation that several of the biochemical species important in the atherosclerotic
25 process, including cholesterol and calcium hydroxyapatite, can be easily detected below the tissue surface, we wished to determine the depth limit of detection using the NIR FT Raman technique. In order to address this question, ten 200 μm
30 sections of aortic media were cut and placed one at a time over a large calcified deposit (6x6x3 mm), and the FT Raman spectra of the 960 cm^{-1} band

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monitored as a function of depth below the surface. As indicated by the plot of FT Raman intensity versus depth shown in Figure 7, the signal from the calcified deposit was detectable until the deposit was greater than 1.6 mm below the irradiated surface. Even slightly deeper depths could be probed if the focus of the collection optics was moved into the tissue.

The two dimensional resolution of the NIR FT Raman signal for material below the tissue surface was then tested by placing 1 mm of aortic media above another calcified deposit, and moving the tissue transversely in two dimensions through the laser beam and collection lens. The FT Raman signal was observed to drop-off rapidly as the beam and collection optics moved from the calcified deposit. The detected FT Raman signal closely followed the geometry of the calcified deposit below the surface, despite the significant scattering of the overlying layer of tissue. This result suggest that the Raman scattered light may be utilized for imaging objects below the tissue surface with minimal image blurring due to elastic scattering in the tissue.

A second spectroscopic method is also used to obtain molecular vibration information, attenuated total reflective (ATR) of infrared light.

Human aorta was chosen as an example to illustrate the diagnosis of atherosclerotic artery tissue. As in the samples obtained for the Raman spectral measurements human aorta samples were obtained for ATR measurements at the time of post mortem examination. Sample storage and preparation procedures are identical to those set forth for the

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Raman spectral measurements. These reflectance measurements can be used by themselves to provide diagnostic data in conjunction with either the Raman spectroscopic measurements described above or with
5 fluorescence measurements, or with both types of measurements to enhance diagnosis for specific applications.

The medial layers of a normal arteries and the necrotic cores of atheromatous plaques were exposed
10 by blunt dissection and spectroscopically examined. ATR spectra were also collected from several purified tissue components including collagen, elastin, and cholesterol to assist in analysis of the spectra.

15 Mid-infrared ATR spectra were measured from 4000 to 700 cm^{-1} with a commercially available FT-IR spectrometer and a horizontal ATR accessory. The sampling area was purged with dry nitrogen gas to control background absorption from atmospheric water
20 vapor and CO_2 . Spectra were collected at 4 cm^{-1} resolution with 50 scans. The artery specimens, kept physiologically moist with isotonic saline (buffered at pH 7.4), were placed in contact with the ATR element (ZnSe crystal 45 ends). A 5 gram
25 weight placed on the tissue sample ensured uniform sample contact with the ATR element. An ATR spectrum of the saline solution with absorbance similar to that of the artery samples was also obtained and used for subtraction of spectral
30 components due to water. Collagen (Calbiochem: type I, bovine achilles tendon) and elastin (Sigma: bovine neck ligament) were prepared as saline

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slurries. Cholesterol (Sigma) was prepared as a dry film on the ATR element by evaporation of a benzene solution. These elements can be clearly identified in the resulting spectra.

5 The ATR sampling crystal is a rod of high refractive index material which acts as a waveguide for the infrared sampling beam. This waveguide can be in the form of a needle that is adapted for penetration into the tissue to be diagnosed.

10 Alternatively, the probe will have a geometry suitable for contacting the surface of exposed tissue sites or for contacting internal locations with a catheter.

 The devices shown in Figures 16A and 16B
15 illustrate preferred embodiments of the invention adapted for ATR diagnostic measurements within the human body. In Figure 16A a single-ended probe 100 is shown where one or more optical fibers 102 both the incident light to, and the transmitted
20 (reflected) light from, the ATR element 104. A 100% infrared reflector 106 such as gold is placed at the distal surface 108 of the ATR element 104 functions to return the transmitted light back through the same fiber as well as to provide double pass
25 sampling. The ATR element 104 can be a separate component optically fastened to the optical fibers 102, or alternatively, it can be constructed from the end of the optical fiber by removing the cladding material. Sampling is provided by placing
30 the ATR element in contact with the tissue 110 of interest. Radiation is transmitted 112 and collected 114 in a radial direction from element 104. The probe can either be inserted through a

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standard endoscope or catheter to sample a hollow organ, or, if made with sufficiently thin optical fiber, it can be directly inserted directly into a solid organ as in the case of needle biopsy. In this particular embodiment the distal tip 108 is in the form of a needle. The cone or needle configuration on the end of the catheter can be long or shallow.

A double-ended probe is illustrated in Figure 16B. Incident IR beam from FT-IR is transmitted through IR optical fiber 122 to ATR element 128 positioned at the distal end of catheter body 120. The ATR element is placed in contact with tissue 126 surface to be sampled. Transmitted light is conducted through a second IR optical fiber 124 back to an IR detector. The ATR element may be a separate component optically fastened to the two optical fibers 122, 124, or it may be simply a region of a single optical fiber in which the fiber cladding material has been removed. The entire apparatus can be inserted through a standard endoscope or outer catheter.

For methods of measuring excised samples, the specimen to be sampled is placed in optical contact with the surface of the waveguide or ATR element. The evanescent wave which extends outside of the waveguide surface is absorbed by the sample in proportion to its absorption coefficient. The penetration depth of the evanescent wave into the sample depends on the wavelength of the infrared radiation and the refractive indices of the waveguide and the sample; for a ZnSe-water interface, this depth is roughly 1 μm from 1800 to

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700 cm^{-1} . The $1/e$ penetration depth of the evanescent wave into the sample is given by $\lambda/2\pi(n_z^2\sin^2\theta - n_w^2)^{1/2}$, where λ is wavelength, θ is angle of incidence and n_z and n_w are the refractive indices of ZnSe and water respectively. Consequently, only tissue that is in good optical contact with the ATR element will be sampled. In addition, individual components in the sample can exhibit different affinities for the waveguide material (ZnSe in this case), which can influence the relative concentrations of the components at the waveguide surface. Despite relatively high concentrations in the bulk tissue, components with poor optical contact can be difficult to measure in the ATR spectrum.

Figure 8 shows FT-IR ATR spectra of (a) normal aorta (intimal side) and (b) buffered saline. A comparison of these spectra shows that a majority of the IR absorption of normal intima can be attributed to water, which comprises roughly 80% of the tissue by weight. The large, broad bands peaking at 3300 cm^{-1} and 1636 cm^{-1} are due to the O-H stretching and H-O-H bending vibrations, respectively, of water, and the weak band at 2120 cm^{-1} is due to a water combination vibration. The 3300 cm^{-1} and 1636 cm^{-1} bands also include contributions from the N-H stretching and amide I vibrations. The relatively flat absorption between 1500 and 900 cm^{-1} and the rising absorption below 900 cm^{-1} is also due primarily to water; however, in the intima, a number of very weak bands due to other tissue components are also present in this region.

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Most biomolecules give rise to IR absorption bands between 1800 and 700 cm^{-1} , which is known as the "fingerprint region" or primary absorption region. The dominant absorption of tissue water in this region obscures the absorption bands from other tissue components. To observe the IR bands from these components, one must eliminate the water interference. With the ATR method, spectral deconvolution or subtraction of the water component is particularly easy. By using the 2120 cm^{-1} band, which is due solely to water, as an internal intensity standard the spectrum of buffered saline (Figure 8b) can be accurately and reliably subtracted from the spectrum of aorta intima (Figure 8a), yielding a water-subtracted spectrum of intima (Figure 9a).

In the water-subtracted spectrum, the previously weak bands are easily observed. Band assignments, based on the spectra of the major tissue components are listed in Table I. Most of the vibrational bands observed in the spectrum of normal intima (Figure 9a) can be divided into two broad categories: lipid-associated bands and protein-associated bands. All of the strong bands in normal intima are associated with one of these moieties (see Table I). This can be seen as a simple consequence of the large concentrations of these two materials. Aside from water, a large fraction of tissue can be divided into one of these two groups. Moreover, both protein and lipid components contain repeating molecular units which are common to all members of the group. For

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Table I. Preliminary assignments of IR absorption peaks in the ATR spectra of normal aorta intima.

V (+1cm ⁻¹)	Preliminary Vibrational Assignment	Associated Tissue Components
2923(s)	C-H stretch	Lipid, Protein, Others
2853(s)	C-H stretch	Lipid, Protein, Others
1744(s)	C=O (ester) stretch	Lipid
1651(s)	Amide I	Protein
1635(sh)	Amide I, H-O-H bend	Protein, Water
1548(s)	Amide II	Protein
1465(s)	CH ₂ bend	Lipid
1457(s)	CH ₂ bend, CH ₃ anti-symmetric deformation	Lipid
1454(s)	CH bend, CH ₃ anti-symmetric deformation	Protein, others
1417(w)	CH ₂ bend adjacent to C=O	Lipid
1401(m)	COO ⁻ symmetric stretch, amide C-N stretch	Protein, others
1378(w)	CH ₃ symmetric deformation	Lipid
1244(m)	Amide III, PO ₂ ⁻ anti-symmetric stretch	Protein, others
1239(m)	CH ₂ wag, PO ₂ ⁻ anti-symmetric stretch	Lipid
1159(s)	CH ₂ wag, C-O-C antisymmetric stretch	Lipid
1117(w)	C-C stretch, O-C-O stretch	Lipid
1096(w)		Lipid
1083(w)	PO ₂ ⁻ symmetric stretch	Protein, others
1030(w)		Lipid
965(w)	C=CH deformation (trans)	Lipid
722(m)	CH ₂ rock	Lipid

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Table II. Peak frequencies of selected bands in normal and atherosclerotic aorta.

Normal	Adventitia'	Fibrous Plaque	Fatty Plaque	Exposed Calcif. I	Exposed Calcif. II	Assignments
	1746w					C=O (ester) stretch
	1667m	1667m		1667m		C=C Stretch Lipid
1658s				1663m		Amide I (8)
	1655m					C=C stretch Fatty Acids
		1519w				Carotenoid (12)
1451s	1440s	1440s	1440s	1450s	1440s	C-H bend (8) Protein Lipid
	1301m 1267w	1301w 1264w	1301w 1262w		1300w 1262w	Lipid C-H bend (CH ₂) Lipid C-H bend (=C-H)
1252m				1261w		Amide III (8)
		1157w				Carotenoid (12)
	1080m	1131w 1086w	1130w 1088w	1128w		C-C stretch Lipid

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Table II. Continued

Normal	Adventitia*	Fibrous Plaque	Fatty Plaque	Exposed Calcif.I	Exposed Calcif.II	Assignments
				1071s	1071s	Phosphate antisymmetric stretch Calcium salts (15)
1004w		1004w	1004w			Phenylalanine (8)
				960vs	960vs	Phosphate symmetri stretch Calcium salts (15)
		957w 882w 842w 803w 700m 606w 546w	956w 882w 841w 801w 700m 606w 546w		878w 850w 804w 699m 547w	Cholestereols (11)
				587m	587m	Phosphate Calcium salts (15)

Peak frequencies of typical specimens, accurate to $\pm 1 \text{ cm}^{-1}$. Abbreviations: vs=very strong, s=strong, m=medium, and w=weak relative band intensity.
 *Adventitia specimen is mainly adipose tissue.

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protein, the polypeptide backbone of repeating amide groups is the dominant element. In lipids, the repeating hydrocarbon chain is the defining quality. The end result is that these molecular units are present in very large concentrations, and their vibrational bands tend to dominate the spectrum. Note that this does not imply that no further level of detail is derivable from the IR spectrum of tissue. For example, the frequencies of the amide group vibrations are sensitive to protein configuration and conformation. Therefore, shifts in protein makeup might be expected to produce observable changes in the amide bands.

The water-subtracted spectrum of sub-adventitial fat shown in Figure 9b, more clearly illustrates the division of bands into lipid and non-lipid categories. This fat can be considered as the model of the lipid component. Protein contributions, as judged from the intensities of the amide I and II bands, are negligible for the purposes of this model. All of the bands observed in the fat spectrum can be attributed to the lipid component. These include the strong bands at 1744 cm^{-1} (C=O stretch), 1465 cm^{-1} (C-H bend), 1161 cm^{-1} (CH_2 wag, C-O-C stretch), as well as the weaker bands at 1378 cm^{-1} , 1239 cm^{-1} , 1118 cm^{-1} , 1099 cm^{-1} , 966 cm^{-1} , and 722 cm^{-1} .

The bands observed in the water-subtracted spectrum of intima constitute less than 30% of the total absorption, the rest being due to water. Any conclusions regarding these relatively weak bands depends critically upon the accuracy of the water

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subtraction. The accuracy of this subtraction can be judged from the reproducibility of spectra obtained sequentially from the same sample. Two consecutive water-subtracted spectra collected 10 minutes apart from a sample of normal aorta (intimal side) are shown in Figure 10a (solid and dashed curves). Most of the IR bands exhibit a substantial increase in absorbance with time. This trend continues for consecutive spectra collected more than an hour after the placement of the sample on the ATR element. However, not all of the bands change by the same fraction, so that the relative intensities differ between consecutive spectra. For instance, in Figure 10a, the C=O band at 1744 cm^{-1} is relatively constant, while the amide bands at 1650 cm^{-1} and 1547 cm^{-1} increase by 50% in the later spectrum. Although these changes might seem to indicate that the water subtraction is inaccurate, the changes with time are systematic and predictable, which suggests that the optical contact between the sample and the ATR element is changing regularly with time.

In fact, the constancy of the 1744 cm^{-1} C=O band, which is due solely to lipid, and the increases in the amide bands, which are due to protein, indicate that the lipid contributions to the IR absorption remain unchanged while the non-lipid contributions increase between consecutive scans. This is confirmed by subtracting the spectrum of lipid (Figure 9b) from the water-subtracted spectra of aorta intima (Figure 10a), using the 1744 cm^{-1} band for normalization. The

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resulting lipid-subtracted spectra of aorta intima are shown, normalized to peak absorbance, in Figure 10b. As can be seen, the relative peak absorbencies and spectral bandshapes in the lipid-subtracted spectra reproduce quite well, reflecting the accuracy of both the water and the lipid-subtraction procedures.

The constancy of the lipid bands and the variation of the non-lipid bands between successive scans may seem somewhat puzzling. An explanation of this apparent anomaly can be inferred from a water-subtracted spectrum of saline solution which is rinsed off the surface of the tissue (Figure 9c). This spectrum, aside from the weak amide I and II bands, matches quite closely with that of adventitial fat. The lipid component observed in the tissue appears to be due to free lipid particles that have equilibrated with the tissue surface water, forming a thin water-lipid film on the tissue surface which is in full optical contact with the ATR element immediately after the tissue specimen is placed upon the crystal. The tissue components beneath this film presumably achieve better optical contact with the ATR crystal as the sample settles. As a result, the content of lipid in a spectrum of aorta intima or media may be influenced by the presence of sub-adventitial fat in the specimen, and the relative lipid-protein absorbencies are accurate to 50% at best with the present experimental design. For the reason, all of the remaining spectra shown are both water and lipid subtracted.

With the lipid bands removed, assessment of the non-lipid bands in the spectrum of normal intima

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(Figure 10b) is greatly simplified. The major bands in the spectrum may be assigned to protein backbone vibrations. These include the bands at 1648 cm^{-1} (amide I), 1549 cm^{-1} (amide II), 1455 cm^{-1} (C-H bend), 1401 cm^{-1} (amide C-N stretch), and 1244 cm^{-1} (amide III). The frequency of the amide I peak (1648 cm^{-1}), which is sensitive to protein secondary structure, may indicate contributions from α -helix, disordered, and collagen helix conformations. This band also exhibits a shoulder near 1634 cm^{-1} , which may be due to the β -sheet regions of proteins or water. The protein C-H bending band at 1455 cm^{-1} is distinct from the corresponding vibration in lipid, which occurs as a double-peaked band at 1465/1457 cm^{-1} . Note that all of these bands may include contributions from other moieties. For instance, the symmetric stretch of carboxylate groups and the antisymmetric stretch of phosphate groups may also contribute, respectively, to the 1401 cm^{-1} and 1244 cm^{-1} bands. This correlation of components is summarized in Table I above.

A typical spectrum of the medial layer of normal aorta is shown in Figure 11a. A comparison of this spectrum to that of normal intima (Figure 10b) fails to reveal any significant differences. This result is somewhat surprising, considering that normal aorta intima and media have significantly different compositions. Typical spectra of an atherosclerotic plaque and a non-ulcerated atheromatous plaque are shown in Figures 11b and 11c, respectively. For these plaques, only the intact fibrous cap at the intimal surface is probed

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due to the short penetration depth ($1\ \mu\text{m}$) of the beam. Any necrotic, atheromatous material beneath this fibrous cap is not sampled. Even so, the fibrous caps of these plaques are known to be compositionally different than normal intima and one might expect these differences to be reflected in the IR ATR spectrum. However, as in the case of media, no consistent differences are observed in the spectra of these plaques (Figures 11b and 11c) and normal intima (Figure 10b). This issue will be expanded upon in the discussion below.

On the other hand, substantial differences are obvious in the spectrum of the necrotic, atheromatous core of an atheromatous plaque (Figure 12a) as compared with the corresponding spectra of normal intima (Figure 10b) as well as those of intact atherosclerotic (Figure 11b) and atheromatous (Figure 11c) plaques. In this case, the necrotic core was presumably exposed in vivo as disease progressed by ulceration of the overlying intimal fibrous tissue cap. (The spectrum of necrotic core exposed by dissecting away the fibrous cap of a non-ulcerated atheromatous plaque is similar.) A new band appears at $1050\ \text{cm}^{-1}$, with a secondary peak at $1023\ \text{cm}^{-1}$. In addition, the necrotic core spectrum exhibits an increase and frequency shift in the $1466\ \text{cm}^{-1}$ band as compared with the $1455\ \text{cm}^{-1}$ protein band in normal intima as well as a set of unique bands near $1382\ \text{cm}^{-1}$. These characteristic bands are found in the spectra of all the exposed necrotic core samples and in none of the other samples (see below).

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The source of these unique bands in the necrotic core spectra may be cholesterol, which is known to accumulate in large amounts in atheromatous cores. An ATR spectrum of cholesterol (dry film) is shown in Figure 12b. The three major bands unique to the necrotic core, near 1463 cm^{-1} , 1382 cm^{-1} , and 1050 cm^{-1} , match closely in position and relative intensities with the three main cholesterol bands at 1466 cm^{-1} , 1377 cm^{-1} , and 1056 cm^{-1} . Each of the main cholesterol bands has a secondary peak, which also appear to be present in the necrotic core bands. These secondary peaks occur at $1445/1436\text{ cm}^{-1}$, and 1023 cm^{-1} in the cholesterol spectrum and at 1441 cm^{-1} , 1367 cm^{-1} , and 1023 cm^{-1} in the necrotic core spectrum. In addition, several of the weak bands in the necrotic core spectrum, including the peaks at 1334 cm^{-1} , 1109 cm^{-1} , 954 cm^{-1} , and 797 cm^{-1} , are associated with the weaker cholesterol bands near these frequencies. Other components in the necrotic core may also contribute to some of these distinct bands.

The consistency of the spectral differences which are attributed to cholesterol between the necrotic core specimens and the normal, atherosclerotic, and non-ulcerated atheromatous specimens are illustrated in the scatter plot in Figure 13. This plot depicts the integrated intensities (areas) of the 1050 cm^{-1} cholesterol band ratioed to the total protein content, as measured by the area of the amide II band at 1548 cm^{-1} . The 1050 cm^{-1} band was integrated from 1075 to 1000 cm^{-1} and baseline subtracted using these endpoints, and the

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amide II band was integrated from 1593 to 1485 cm^{-1} with a similar baseline subtraction. This ratio is a measure as the relative cholesterol contribution to the spectrum, and is proportional to the relative cholesterol concentration of the sample with the assumption that the area of the 1050 cm^{-1} band is due solely to cholesterol. As can be seen in Figure 13, this ratio is higher for all the exposed necrotic core specimens than for all the other specimens.

The consistent results of this sample analysis, which is possible because of the separation and molecular identification of the bands, indicates the potential of IR spectroscopy for tissue characterization.

Investigations of human arteries and atherosclerosis by mid-IR spectroscopy have been limited to date. It has been reported that ATR spectra have been recorded from partially dried human artery, among other tissues. In comparing a normal aorta from an infant to an atherosclerotic plaque in an adult, they observed increases in several bands in the atherosclerotic aorta. Most of these bands were associated with lipids and lipoproteins. IR spectroscopy has been employed to determine the chemical composition of calcified atherosclerotic deposits. A more detailed IR study of atherosclerotic aorta involves recorded IR transmission spectra from thin layers sectioned at different depths into the arterial wall. Results showed increased absorption near 1739 cm^{-1} in the fatty (atheromatous) regions of plaque, which was attributed to absorption by cholesterol esters in

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the plaque. IR spectra from the fibrous tissue cap at the surface of the plaques were similar to normal intima.

One of the main difficulties in measuring mid-
5 infrared spectra of intact human tissue is the intense water absorption, which dominates and obscures the absorption of other tissue components of interest. In most of the studies cited above, the water absorption was not eliminated, limiting
10 the quality and amount of information available from the spectra. With the ATR sampling method, this water interference is easily removed (see Figure 9). The ATR method is also naturally amenable to sampling with fiber optic probes in vivo. Water
15 interference in fiber optic probe ATR spectra of aqueous protein solutions has been accurately eliminated with a water subtraction procedure similar to the one employed in the present study.

While the ATR method is well suited to in vivo
20 sampling and to accurate subtraction of the water signal, spectra collected with the ATR method are not equivalent to IR absorption spectra, but depend on properties of the ATR material and the sample in addition to the sample absorption coefficient. For
25 instance, the penetration depth of the evanescent sampling wave depends on the refractive indices of the ATR material and the sample. However, the refractive indices of both ZnSe and human tissue are expected to vary slowly with frequency between 1800
30 and 700 cm^{-1} and such variations will at most affect the relative intensities of bands at different frequencies. All of the structure observed in the

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tissue spectra is attributed to absorption bands in the tissue.

The component absorptions observed in an ATR spectrum also depends upon the optical contact of the sample and ATR element. The small penetration depth of the evanescent wave into the tissue sample implies that only a 5 μm thick layer, and preferably about 1 micron, of material at the surface is observed. This is referred to as the near surface region of the tissue for the purposes of this application. The tissue deeper than 5 microns from the surface is defined as the sub-surface region. This thin, sampled near-surface layer may differ in composition with the bulk sample. For example, a film of free water may be present on the surface of wet tissue, with different levels of some molecular species of the tissue relative to their concentrations in the bulk tissue. In addition, the varied affinities for the ATR material of different moieties in the tissue may play an important role in the intensities of the observed bands.

These considerations may explain the lack of substantial differences among the ATR spectra of normal intima, plaque fibrous cap, and media. For instance, normal aorta intima is composed of roughly 25% collagen (dry weight) and 20% elastin, while aorta media has 20% collagen and 50% elastin. The ATR spectra of purified collagen and purified elastin (not shown) differ substantially. In particular, amide I/II occur at 1657/1556 cm^{-1} in hydrated collagen (type I) and 1653/1543 cm^{-1} in hydrated elastin (spectra not shown).

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One might expect these differences to be reflected in the intima and media ATR spectra. A possible explanation of why this is not the case is that the thin layer in optical contact with the ATR element is compositionally different from the bulk tissue, and collagen and elastin make only a minor contribution to the IR ATR bands of this layer. Such an effect may also explain the lack of significant differences among the plaque fibrous cap intima and normal intima ATR spectra. In ATR elements made of other substances with different biochemical affinities, the spectral differences among these tissues can be substantially enhanced depending on the tissue type.

The results of the present investigation demonstrate that high quality water-subtracted spectra can be readily obtained from human tissue with the ATR technique. Similar results have been obtained in other mammalian tissues. Accurate removal of the water interference is crucial to isolating the relatively weak tissue absorption bands of lipid, protein, as well as other tissue components. It is worth noting that the observation of these relatively weak bands via spectral subtraction depends entirely upon quality of the tissue and saline spectra. For instance, the absorbance of the normal intima specimen (Figure 8a) between 1500 and 900 cm^{-1} is approximately 0.06. In the water-subtracted spectrum (Figure 9a), the peak absorbencies range from 0.018 (30%) for the strongest bands to 0.003 (5%) for the weakest ones. The detection of a 0.003 absorbance peak in a

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subtracted spectrum with a 0.06 absorbance background requires a signal-to-noise ratio of 700 or better in the 100% baseline. Such a signal-to-noise is easily achieved with an FT-spectrometer.

- 5 The high linearity, baseline stability, and wavelength precision of the FT-spectrometer are also obviously critical for accurate background subtraction.

- 10 While water subtraction is relatively easy and accurate with ATR, it may be substantially more difficult with other clinically applicable sampling techniques such as diffuse reflectance or photoacoustic sampling. These alternative sampling techniques are clinically useful, however, because
15 of their longer tissue penetration depths (approximately $10\mu\text{m}$). As an alternative to water subtraction, one can exploit the properties of the spectral lineshape of water to eliminate the water signal by other computational methods.
20 Specifically, the spectral lineshape of water varies rather slowly with frequency over much of the region of interest, especially between 1500 and 700 cm^{-1} . Therefore, any method which filters this slower variation and spares the sharper features of the
25 non-water bands can separate the water and non-water components.

- One such method is second derivative spectroscopy. Differentiation of a spectrum is typically used to narrow absorption bands and
30 resolve overlapping peaks. Differentiation also tends to deemphasize broad bands relative to sharper ones. In IR spectra of artery, the broad,

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featureless absorption of water can be nearly eliminated in favor of the sharper non-water bands by computing the second derivative of the spectra. This is clearly demonstrated in Figure 14, which shows the second derivative of a spectrum of normal aorta intima (Figure 14a), along with the water-subtracted spectrum of the same specimen (Figure 14b). Essentially only the 1633 cm^{-1} water band is left, partially obscuring the amide I band. Elsewhere in this spectrum, the water contribution is minimal. All of the bands identified in the water-subtracted spectrum are easily observed in the second derivative spectrum.

In addition to elimination of water interference, several of the unresolved double peaks and shoulders in the water-subtracted spectrum appear as distinct peaks in the second derivative spectrum. For example, the amide II band in normal intima (Figure 14b) has a very weak shoulder near 1518 cm^{-1} , and the C-H bending region near 1468 cm^{-1} appears to include two overlapping peaks. In the second derivative spectrum (Figure 14a), the 1518 cm^{-1} band is clearly visible, and the C-H region exhibits two separate peaks at 1469 and 1456 cm^{-1} . Moreover, by sharpening the bands, the second derivative spectrum allows a more precise determination of peak frequencies, so that relatively small frequency shifts are observed. Such frequency shifts can be of importance in detecting and characterizing subtle molecular alterations involved in certain tissue conditions.

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The observation of individual, resolved bands in the artery IR ATR spectra is of considerable interest, since separation of bands is the first step determining the composition of a sample from its spectrum. Once a band has been isolated, its integrated intensity is proportional to the concentration of the moiety responsible for that band. In particular, since the amide I and II bands are due entirely to protein, these bands can be used to isolate the overall protein content in the spectrum. The sharp, well resolved 1744 cm^{-1} C=O ester band appears to be due to solely to lipid, and the integrated intensity of this band should be proportional to the relative lipid content are technique should largely eliminate the inaccuracies. Finally, it should be remembered that the relative water content of the tissue sample is automatically computed from the 2120 cm^{-1} band in the water subtraction algorithm. However, as noted earlier, the composition of tissue as determined from an ATR spectrum may not be precisely identical to the composition of the bulk tissue.

The tissue composition can also be determined from overlapping bands by first deconvolving the bands of interest into their individual components. This is especially easy if one component has an additional, isolated band elsewhere in the spectrum. An example is the 1465 cm^{-1} C-H bending region, which is due to different tissue components with distinct spectral features in this region. In the normal intima spectrum (Figure 9a), this band is attributed to a combination of lipid and protein components.

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Since the lipid component also exhibits the isolated 1744 cm^{-1} band, this band can be used to subtract the lipid C-H bending component and isolate the protein C-H bending component at 1455 cm^{-1} (Figure 10b),
5 effectively deconvolving this band. Note that this deconvolution depends on having a reliable spectrum of one of the individual components, which, in this example, is the lipid spectrum in Figure 9b.

The detection of distinct bands attributed to
10 cholesterol in necrotic core may provide a useful means of determining cholesterol concentrations in vivo. Both the 1050 cm^{-1} and 1382 cm^{-1} cholesterol bands appear to be fairly isolated in the necrotic core spectrum after lipid-subtraction (Figure 12).
15 If these two bands are due to a single component, namely cholesterol, the ratio of their integrated intensities should be a constant for all the samples. The baseline-subtracted area of the 1050 cm^{-1} band, $A(1050)$, is plotted versus that of the
20 1382 cm^{-1} band, $A(1382)$, for all the samples, normalized to the protein content in Figure 15. As can be seen in the plot, there is a roughly linear relationship between $A(1050)$ and $A(1382)$. A linear least squares fit to this data yields the line shown
25 in the plot, with a high regression coefficient of $r=0.967$. The slope of this line 2.8, while the ratio $A(1050)/A(1382)$ for the pure cholesterol ATR spectrum is 2.3. The reasonable agreement between these two numbers provides additional evidence for
30 the assignment of these bands to cholesterol. Moreover, it indicates that the relative spectral content of cholesterol is reasonably approximated by

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the integrated intensities of either of these bands. Figure 15 also shows that the ATR spectra of all the specimens other than exposed necrotic core exhibit almost no intensity in both the 1050 and 1382 cm^{-1} bands, in contrast to the necrotic specimens, all of which have significant bands at both frequencies.

The present systems and methods demonstrate that infrared spectra of moist, bulk tissues can be reliably obtained with the ATR technique. Although water is the dominant absorber throughout much of the mid-infrared region, the high quality spectra acquired with the FT-IR ATR technique allow for accurate subtraction of the water signal. Elimination of the water interference is critical for identifying and assigning the absorption bands of other tissue species. The isolation and designation of these relatively sharp bands provides a means of analyzing spectroscopically the composition of arterial tissue non-destructively. There methods are also applicable to the study and diagnosis of other tissues and tissue conditions, such as neoplasia.

The observation of both lipids and cholesterol in the spectra of necrotic atheromatous core samples is particularly exciting, because lipids and cholesterol are thought to play major roles in the pathogenesis of atherosclerosis. The spectral observation of these components, cholesterol in particular, provides a reliable means of detecting and characterizing advanced atheromatous plaques in which ulceration of the fibrous cap has occurred (as demonstrated in Figures 13 and 15). Intimal

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accumulations of lipid and cholesterol occur early in the atherogenic process. Therefore, the mid-IR ATR technique can also be useful in detecting and studying the early fatty streak lesion.

5 Spectrograph/CCD System for NIR Raman Spectra

NIR Raman spectroscopy using a single stage spectrograph and a charge coupled device (CCD) detector offers superior sensitivity over the Nd:YAG excited FT-Raman system of Figures 1A and 1C. By
10 shifting the wavelength of the laser excitation from 1064 nm to the 800-900 nm region, a CCD can be used to detect the Raman scattered signals while still avoiding fluorescence excitation in most molecules. The system can operate usefully in the range of 750
15 nm to 1050 nm. Although the fluorescence emission from tissue is significantly higher with 810 nm than with 1064 nm excitation, the Raman signals are readily observed. This is because the dominant noise source in the spectrograph/CCD system is shot
20 noise associated with the fluorescence emission, which is 2-3 orders of magnitude smaller than the dark current noise of the InGaAs detector, which is the dominant noise source in the FT-Raman system.

Figure 17 shows the laser diagnosis and
25 treatment system of Figure 1A modified to use the spectrograph/CCD system of this invention. The diagnostic subsystem 201' includes a single stage spectrograph 310 and charge-coupled device (CCD) detector 312 for collecting near-infrared (NIR)
30 Raman spectra from intact human arterial tissue. With 810 nm laser light excitation, preferably pulsed, the fluorescence emission from human artery

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tissue is sufficiently weak to observe Raman bands more rapidly with the spectrograph/CCD system than with the 1064 nm excited FT-Raman system of Figures 1A and 1C. A method for removing the broadband
5 emission from the spectra by computing the difference of two emission spectra collected at slightly different excitation frequencies was used to enhance observation of the Raman bands. This method relies on the stability, linearity, and low
10 noise characteristics of the CCD detector. The results indicate that high quality NIR Raman spectra can be collected in under 1 second with the spectrograph/CCD system and an optical fiber probe, as compared to 30 minutes with the FT-Raman system
15 at similar laser power levels, further improving the use of the technique for *in vivo* clinical applications.

A preferred embodiment of a spectrograph/CCD system 300 employed for the collection of near
20 infrared (NIR) Raman spectral data from excised tissue samples using a spectrograph and a charge coupled device (CCD) array is illustrated in Figure 18. NIR Raman spectra were measured from 100 - 2000 cm^{-1} below the laser excitation frequency with a
25 single stage imaging spectrograph 310 (Acton Model ARC275, 0.25 m, F/3.8) and a CCD array 312 (Princeton Instruments EEV Model 88130).

System 300 can use a NIR 810 nm Nd:YAG pumped pulsed dye laser 314 operating at 10 Hz for
30 irradiating a sample 46. Alternatively, a CW or diode laser source can also be employed. Laser 314 generated a laser beam 316 which is directed by

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mirror 318 through focusing optics 320 to impinge on sample 46 mounted behind a transparent window 321. The laser beam was focused on the sample at a 70° angle of incidence, yielding a spot size of 0.7 x 2 mm on the tissue surface. The average incident power at the sample was maintained at 20 mW to avoid excessive peak intensities during an individual pulse. The spectral signals were observed to be linear over a range of average incident powers from 2 to 20 mW.

A portion of the scattered light 322 emitted by sample 46 was collected by collecting optics 324 at a 90° angle relative to the incident laser beam. Collecting optics 324 collimates and F/matches the collected light for the spectrograph 310. Prior to entering the entrance slit of the spectrograph 310, the collected light was passed through a series of Schott glass filters 326 which attenuated the elastically scattered component of the collected light. The combined effect of the Schott glass filters provided an optical density of 7 at 810 nm, a transmission of 20% at 850 nm (580 cm⁻¹ from 810 nm), and a transmission of 85% above 900 nm (1200 cm⁻¹).

The spectrograph 310 utilized a 200 μm slit width and a 600 groove/mm grating blazed at 1 μm and could be scanned to provide spectral coverage over different wavelength regions. The 200 μm slit width provided a resolution of roughly 15 cm⁻¹.

The CCD array 312 consisted of 298 (column) by 1152 (row) pixel elements having a total active area of 6.7 mm x 26 mm, with the short axis parallel to

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the slit. The CCD array was cooled to -110°C to eliminate dark current. Each row of pixels was binned to reduce readout noise. Commercially available CCD detectors offer extremely low detector noise and usable quantum efficiencies out to 1050 nm and provide substantial advantages over InGaAs and other NIR detectors. These advantages outweigh the lower throughput of the grating spectrograph, provided that broadband fluorescence interference is not too great with the shorter excitation wavelengths.

Excised human aorta samples 46 obtained at the time of post-mortem examination were rinsed with isotonic saline solution (buffered at pH 7.4), snap-frozen in liquid nitrogen, and stored at -85°C . Prior to spectroscopic examination, samples were passively warmed to room temperature and were kept moist with the saline solution. Normal and atherosclerotic areas of tissue were identified by gross inspection, separated, and sliced into roughly 8 x 8 mm pieces.

The tissue samples 46 were placed in a suprasil quartz cuvette with a small amount of isotonic saline to keep the specimens moist, and with one surface in contact with the transparent window and irradiated by the laser.

Raman spectra were typically measured between 100 cm^{-1} and 2000 cm^{-1} below the laser excitation frequency. Each spectrum was background subtracted to remove the DC offset of the A/D converter of the CCD controller. In addition, hot pixels due to high energy radiation events were removed from the

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recorded spectrum by applying a median filter having a 7 pixel wide window as to each spectrum. Raman frequencies were calibrated with the spectra of benzene and barium sulfate powder and are accurate to $\pm 5 \text{ cm}^{-1}$. The spectra were not corrected for the wavelength dependent response of the filters, spectrograph, and CCD. For each spectrum shown in the following Figures, Raman signals were accumulated for 5 minutes. Substantially shorter collection times can also be used as described herein.

Figure 19A shows the Raman spectra of a normal aorta sample excited with 810 nm laser light and collected with the spectrograph/CCD system 300. In this case, the broadband background emission, which is presumably due to tissue fluorescence, is roughly five times more intense than the strongest Raman bands at 1650, 1451, 1330, and 1253 cm^{-1} . In contrast, the 1064 nm FT-Raman study of normal human aorta shown in Figure 2a exhibited Raman signals with the peak intensities of the strongest bands, amide I at 1650 cm^{-1} and C-H bend at 1451 cm^{-1} , being roughly three times larger than the broadband background emission. However, this background emission in the spectrograph/CCD system is relatively weak with respect to the Raman signals (i.e., on the order of the Raman signals) and therefore the shot noise associated with detecting this background emission is substantially smaller than the Raman signals, allowing the Raman bands to be made distinct after the background emission signals are removed through filtering or

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subtraction. The shot noise is typically random noise exhibiting a Poisson distribution and is associated with the detector and/or the background emission itself.

5 In contrast, with visible excitations, the fluorescence background emission from the arterial pathology tissue types described is 3 to 4 orders of magnitude larger than the Raman signals, and the shot noise associated with this stronger background
10 emission completely obscures the Raman bands even after the background emissions are removed. However, certain other types of tissue, e.g., colon and bladder, do not exhibit such high level fluorescence reactions at visible excitation
15 frequencies, and therefore can probably operate with visible excitations.

The signal-to-noise ratio of the spectrum of normal aorta collected with the spectrograph/CCD system 300 with 20 mW incident power and 5 minutes
20 collection time (Figure 19A) is similar to that obtained with the FT-Raman system of Figure 1C with 500 mW incident power and 35 minute collection time. Since the observed spectral signal-to-noise ratios are similar, we estimate that the noise level
25 observed with the CCD detector 312 of Figure 18 is roughly 3400 times less than that observed with the InGaAs detector 42 of Figure 1C. For the InGaAs detector, the major noise source is the shot noise of the dark current, while with the CCD detector the
30 dominant noise source is the shot noise of the broadband tissue emission, as the dark current and readout electrons of the CCD are much smaller than this emission.

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This simple analysis has several important implications. First, since the major noise source encountered with the spectrograph/CCD system is shot noise from broadband emission by the tissue sample, the spectral signal-to-noise ratio is proportional to the square root of the product of incident intensity and the collection time.

The FT-Raman and spectrograph/CCD systems can be compared as follows. For the FT-Raman system, the incident intensity is 640 mW/mm². The quantum efficiency of the InGaAs detector at 1200 nm is 0.7, and the FT-spectrometer throughput is 1.1 mm²sr, and the transmission efficiency of the FT-spectrometer and filters is roughly 0.062. For the spectrograph/CCD system, the incident intensity is 14 mW/mm². The CCD quantum efficiency is 0.15 at 900 nm, the spectrograph throughput is 0.043 mm²sr, and the transmission efficiency of the spectrograph and filters is 0.24. Combining these factors and taking into account the ν^4 dependence of the Raman cross-sections, the signal level measured by the FT-Raman spectrum is estimated to be 3400 times greater than that of the spectrograph/CCD spectrum.

Therefore, if the laser intensity is increased to the level employed in the FT-Raman experiments, the collection time could be reduced by a factor of 40, to 8 seconds, with no change in the spectral signal-to-noise ratio. Second, the noise level can be further reduced by using longer excitation wavelengths which minimize the tissue fluorescence emission. However, such reductions in fluorescence emission must be balanced against the decreasing

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quantum efficiency of the CCD at longer wavelengths, and the optimum excitation wavelength also depends on the fluorescence excitation profile of the tissue. For tissue types that exhibit little
5 fluorescence emission at visible wavelengths, such as colon and bladder tissue, the CCD can be operated at visible or near visible wavelengths to take advantage of increased quantum efficiency of the CCD at these wavelengths. Finally, the throughput of a
10 500 μm core, 0.2 numerical aperture fused silica optical fiber is 0.03 mm^2sr , which is roughly the same as that of the spectrograph/CCD system. This means that the present lens collection system can be replaced with an optical fiber probe, as is required
15 for *in vivo* operation, with no additional loss in signal.

Figure 19A shows that although the shot noise due to the broadband tissue emission is relatively small, the sloping broadband fluorescence emission
20 still obscures the sharper Raman signals and complicates determination of peak frequencies and identification of weak bands. Furthermore, given the complexity of human tissue, it is likely that this broadband emission will be significant
25 throughout the useful range of the CCD. Any quantitative analysis of the Raman bands in Figure 19A requires that this broadband emission be first removed from the spectrum. The standard methods of removing fluorescence emission from Raman spectra
30 utilize mathematical filters, which rely upon the fluorescence emission being relatively featureless. In an alternative method the excitation frequency is

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varied over a narrow range ($10 - 30 \text{ cm}^{-1}$). The Raman band positions vary directly with the excitation frequency, while the fluorescence emission remains fairly constant with such small changes in
5 excitation frequency, allowing it to be efficiently subtracted out. In contrast with mathematical filters, this operation requires no assumptions about the emission lineshape.

To implement this method, the Raman spectrum of
10 the normal aorta specimen is recorded with excitation wavelengths of 810 nm (Figure 19A) and 812 nm. The Raman bands shift with the excitation frequency by 30 cm^{-1} , while the fluorescence emission remains fairly constant. By subtracting these two
15 spectra, the broadband emission is greatly reduced, and the Raman bands are more readily observed (Figure 19B). This operation is mathematically analogous to taking the derivative of the Raman spectrum, so that the original Raman spectrum can be
20 recovered by integrating the difference spectrum, as shown in Figure 19C. The fluorescence background is greatly reduced in Figure 19C as compared with Figure 19A, allowing easier identification of the Raman bands and their peak frequencies. The
25 integration also smooths the Raman spectrum over a bandwidth similar to the excitation frequency shift and causes some linewidth broadening, as is evident from Figure 19C. Note that the accuracy of this method depends upon the high linearity and stability
30 of the CCD array.

The NIR Raman spectrum of an atherosclerotic plaque with a calcified deposit exposed at the

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surface collected with the spectrograph/CCD system is shown in Figure 20A. In this case, the broadband emission is nearly 10 times greater than that observed in normal aorta (Figure 19A), resulting in increased noise. However, the intense phosphate stretching vibration at 960 cm^{-1} , due to the calcified salts, is readily identified. This band is sufficiently intense to be observed in real time and was used in aligning the collection optics.

Some weaker bands may also be identified, such as the phosphate/carbonate band at 1070 cm^{-1} , although these are obscured by the large fluorescence background. By subtracting out this fluorescence (Figure 20B), as above, these bands are much more easily distinguished. The Raman spectrum obtained by integrating the difference spectrum is shown in Figure 20C. The broadband emission is reduced by a factor of 50 relative to Raman bands, and several weaker bands are readily observed. This spectrum is remarkably similar to that of Figure 5a which was observed with the FT-Raman system and 1064 nm excitation.

As another example of the sensitivity of the spectrograph/CCD system 300, the Raman spectrum of adventitial adipose tissue is shown in Figure 21, which can be compared to the FT-Raman spectrum shown in Figure 5c. The broadband emission is similar to that of normal aorta, while the Raman bands, due mainly to triglycerides in the tissue, are very strong, resulting in an excellent spectral signal-to-noise ratio.

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Thus, the spectrograph/CCD system with 810 nm excitation offers a faster alternative to FT-Raman with 1064 nm excitation and which has greater sensitivity. Even in complex mixtures such as human tissue, the level of background emission observed with 810 nm excitation is low enough to observe the Raman signals. This fluorescence emission does not excessively degrade the signal-to-noise ratio. By subtracting two spectra collected at slightly different excitation wavelengths, and then integrating the difference spectrum, this broadband emission is rejected, yielding high quality Raman spectra. Deconvolution techniques can also be used to selectively remove, or reduce, Raman, fluorescence, or noise light components. Improvements such as using a CW laser to increase the incident intensity and a back-thinned CCD having better red response allows Raman spectra to be collected from intact human tissue in under 1 second. Longer excitation wavelengths may reduce the background emission further. Implementation of the spectrograph/CCD system with a high power diode laser and an optical fiber probe will provide a compact, mobile system for rapidly acquiring NIR Raman spectra remotely from human tissues and will provide a powerful tool for in vivo clinical applications.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using routine experimentation, many equivalents to the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

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CLAIMS

1. A spectroscopic diagnostic system comprising:
 - a laser emitting radiation in the infrared spectrum;
 - a fiber optic cable optically coupled to the laser to deliver the infrared radiation to a distal end of the catheter and to collect Raman shifted radiation emitted by the tissue for delivery to a proximal end of the cable; and
 - a spectral analyzer to receive the collected Raman shifted radiation.
2. A method of spectroscopic diagnosis of tissue comprising:
 - irradiating a portion of tissue to be diagnosed with radiation having a frequency within the infrared range;
 - detecting light emitted by the portion of tissue in response to the radiation, the light having a Raman shifted frequency different from the irradiating frequency; and
 - analyzing the detected light to diagnose a condition of the portion of tissue.
3. The method of spectroscopic diagnosis of Claim 2 wherein the detecting step further comprises detecting a plurality of Raman shifted frequencies and analyzing the plurality of shifted frequencies to diagnose the tissue.

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4. The method of spectroscopic diagnosis of Claim 2 further comprising coupling radiation from a radiation source to a fiber optic cable to transmit the radiation onto the portion of tissue.

5. A method for spectroscopic diagnosis of tissue comprising:

selecting an optical waveguide having an index of refraction correlated with the index of refraction of a portion of tissue to be diagnosed;

irradiating the portion of tissue through the waveguide with radiation having a range of frequencies in the infrared spectrum;

collecting light emitted by the tissue in response to the radiation with the waveguide;

transmitting the collected light from the waveguide to a spectral analyzer; and

analyzing the detected light to diagnose a condition of the tissue.

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6. A method of spectroscopic diagnosis of tissue comprising:
 - irradiating a portion of tissue to be diagnosed with laser radiation;
 - detecting light emitted by the portion of tissue in response to the radiation, the light having a Raman shifted frequency component different from the irradiating frequency and further having background light components having shot noise levels below the level of the Raman light component;
 - removing the background light components from the detected light; and
 - analyzing the remaining detected light to diagnose a condition of the portion of tissue.
7. The method of spectroscopic diagnosis of Claim 6 wherein the detecting step further comprises detecting a plurality of Raman shifted frequency components and background light components and analyzing the plurality of Raman shifted frequency components to diagnose the tissue.
8. The method of spectroscopic diagnosis of Claim 7 wherein the detecting step further comprises substantially removing the background light components from the detected light to leave substantially the Raman shifted frequency light components.

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9. The method of spectroscopic diagnosis of Claim 8 wherein

the irradiating step further comprises irradiating a portion of the tissue with a first frequency and then irradiating the same portion of tissue with a second frequency slightly shifted from the first frequency; and

the detecting step further comprises detecting light emitted by the tissue in response to irradiation by the first frequency to generate a first spectrum of emitted light frequency components, detecting light emitted by the tissue in response to irradiation by the second frequency to generate a second spectrum of emitted light frequency components, and generating a difference spectrum from the first spectrum and the second spectrum by subtracting one from the other, the difference spectrum containing substantially the Raman shifted frequency components of the first spectrum and the second spectrum.

10. The spectroscopic diagnosis method of Claim 9 wherein the first and second irradiation frequencies have wavelengths of between 750 nm and 900 nm, and the second frequency is shifted from the first frequency by less than 50 cm^{-1} .

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11. The spectroscopic diagnosis method of Claim 9 wherein the detecting step further comprises generating the first spectrum and the second spectrum of the emitted light frequency components with a spectroscope and detecting the first spectrum and the second spectrum with a charge coupled device.
12. The spectroscopic diagnosis method of Claim 11 wherein the spectroscope comprises a single stage spectroscope.
13. The spectroscopic diagnosis method of Claim 11 herein the difference spectrum is electronically generated from the first and second spectra detected with the charge coupled device.
14. The method of spectroscopic diagnosis of Claim 6 wherein the detecting step further comprises generating a spectrum of the emitted light frequency components with a spectroscope and detecting the spectrum with a charge coupled device.
15. The method of spectroscopic diagnosis of Claim 14 further comprising coupling radiation from a radiation source to a fiber optic cable to transmit the radiation onto the portion of tissue.

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16. The method of spectroscopic diagnosis of Claim 14 wherein the fiber optic cable comprises a catheter for insertion into body lumens.
17. The method of spectroscopic diagnosis of Claim 15 wherein the fiber optic cable receives light emitted by the tissue and transmits the emitted light to the spectroscope.
18. The method of spectroscopic diagnosis of Claim 17 wherein the spectroscope comprises a single stage spectroscope.
19. The method of spectroscopic diagnosis of Claim 14 further comprising an optical needle to which the radiation is coupled for delivery to the tissue.
20. The method of spectroscopic diagnosis of Claim 14 further comprising detecting light reflected by the tissue and analyzing the reflected light to diagnosis the tissue.

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21. A method of spectroscopic diagnosis of arterial tissue comprising:
- positioning a catheter containing a light transmitting fiber optic cable adjacent to a portion of tissue within an artery to be diagnosed;
 - irradiating the portion of tissue with radiation having a frequency within the infrared range;
 - collecting light emitted by the portion of tissue in response to the radiation with the catheter, the light having a Raman shifted frequency different from the irradiating frequency;
 - transmitting the collected light to a proximal end of the catheter; and
 - analyzing the detected light received at the proximal end to diagnose a condition of the portion of tissue.
22. The method of spectroscopic diagnosis of Claim 21 wherein the detecting step further comprises detecting a plurality of Raman shifted frequencies and analyzing the plurality of shifted frequencies to diagnose the tissue.
23. The method of spectroscopic diagnosis of Claim 21 wherein the fiber optic cable receives light emitted by the tissue and transmits the emitted light of a spectroscopic analysis system.

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24. The method of spectroscopic diagnosis of Claim 23 wherein the spectroscopic analysis system comprises a Fourier transform spectrometer.
25. The method of spectroscopic diagnosis of Claim 21 further comprising detecting light reflected by the tissue and analyzing the reflected light to diagnose the tissue.
26. A method for spectroscopic diagnosis of tissue comprising:
 - selecting an optical waveguide having an index of refraction correlated with the index of refraction of a portion of tissue to be diagnosed;
 - irradiating the portion of tissue through the waveguide with radiation having a range of frequencies in the infrared spectrum;
 - collecting light emitted by the tissue in response to the radiation with the waveguide;
 - transmitting the collected light from the waveguide to a spectroscope for generating a spectrum of emitted light frequencies;
 - detecting the spectrum of emitted light frequencies with a charge coupled device; and
 - analyzing the detected spectrum of emitted light frequencies to diagnose a condition of the tissue.
27. The method of spectroscopic diagnosis of Claim 26 further comprising coupling the radiation to the waveguide with a fiber optic cable.

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28. The method of spectroscopic diagnosis of Claim 26 wherein the optical waveguide comprises a needle.
29. A method of spectroscopic diagnosis of tissue comprising:
 - irradiating a portion of tissue to be diagnosed with laser radiation;
 - detecting light emitted by the portion of tissue in response to the laser radiation with a charge coupled device, the light having a Raman shifted frequency component different from the irradiating frequency; and
 - analyzing the detected light to diagnose a condition of the portion of tissue.
30. A spectroscopic diagnostic system for analyzing tissue comprising:
 - a laser emitting laser radiation;
 - a fiber optic cable optically coupled to the laser to deliver the laser radiation to a distal end of the catheter and to collect Raman shifted radiation emitted by the tissue for delivery to a proximal end of the cable; and
 - a spectral analyzer to receive the collected Raman shifted radiation comprising a spectroscope for generating a spectrum of the collected Raman shifted radiation and a charge coupled device for detecting the generated spectrum.

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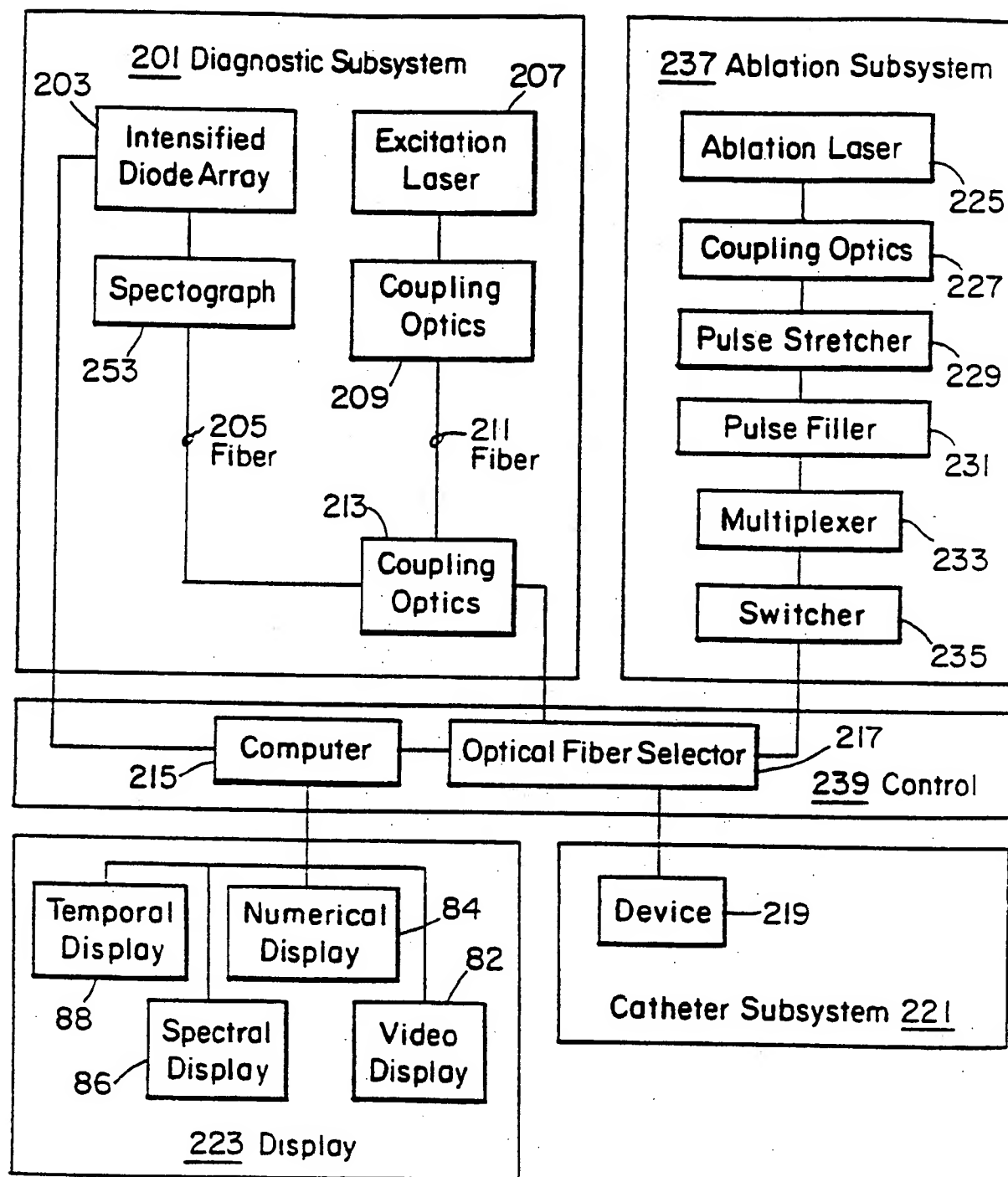


Fig. 1A

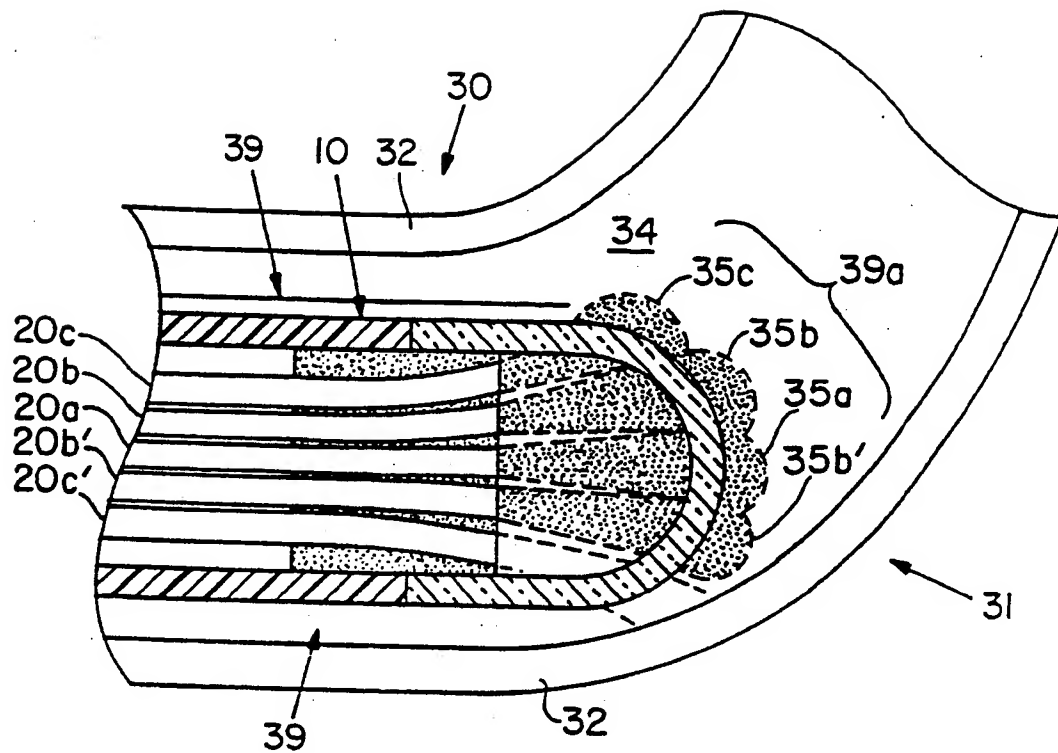


Fig. 1B

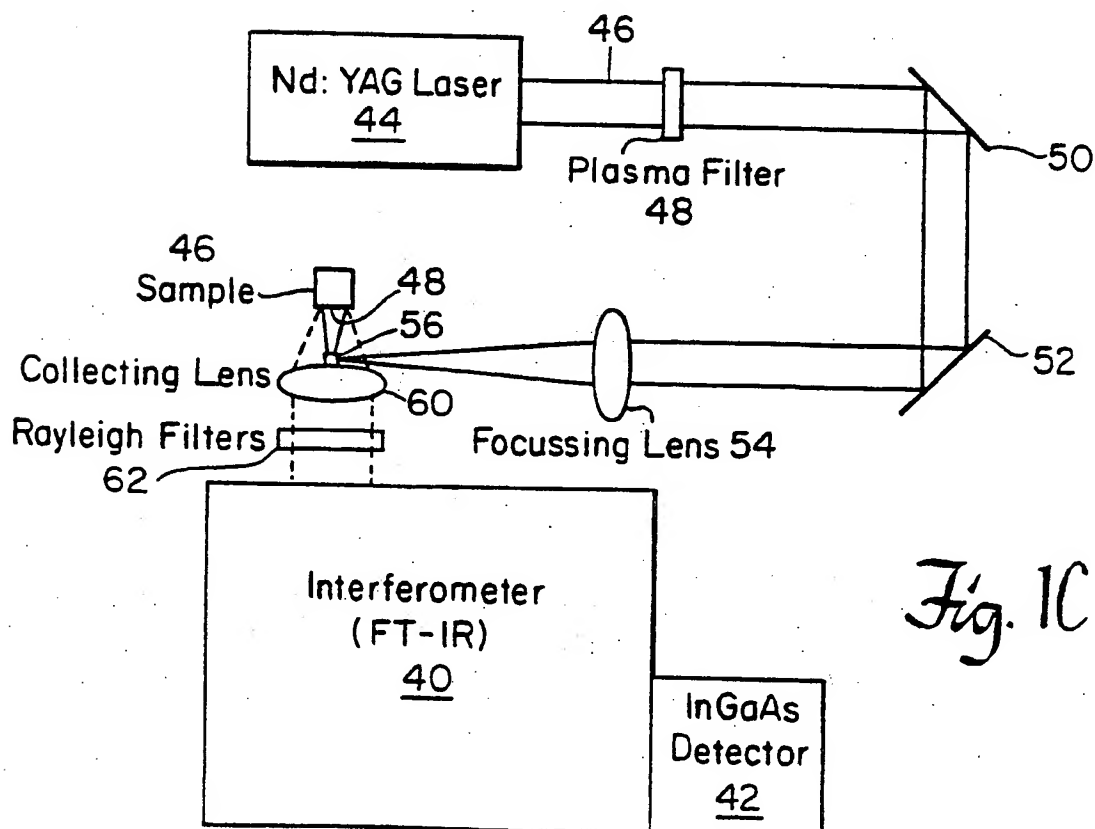
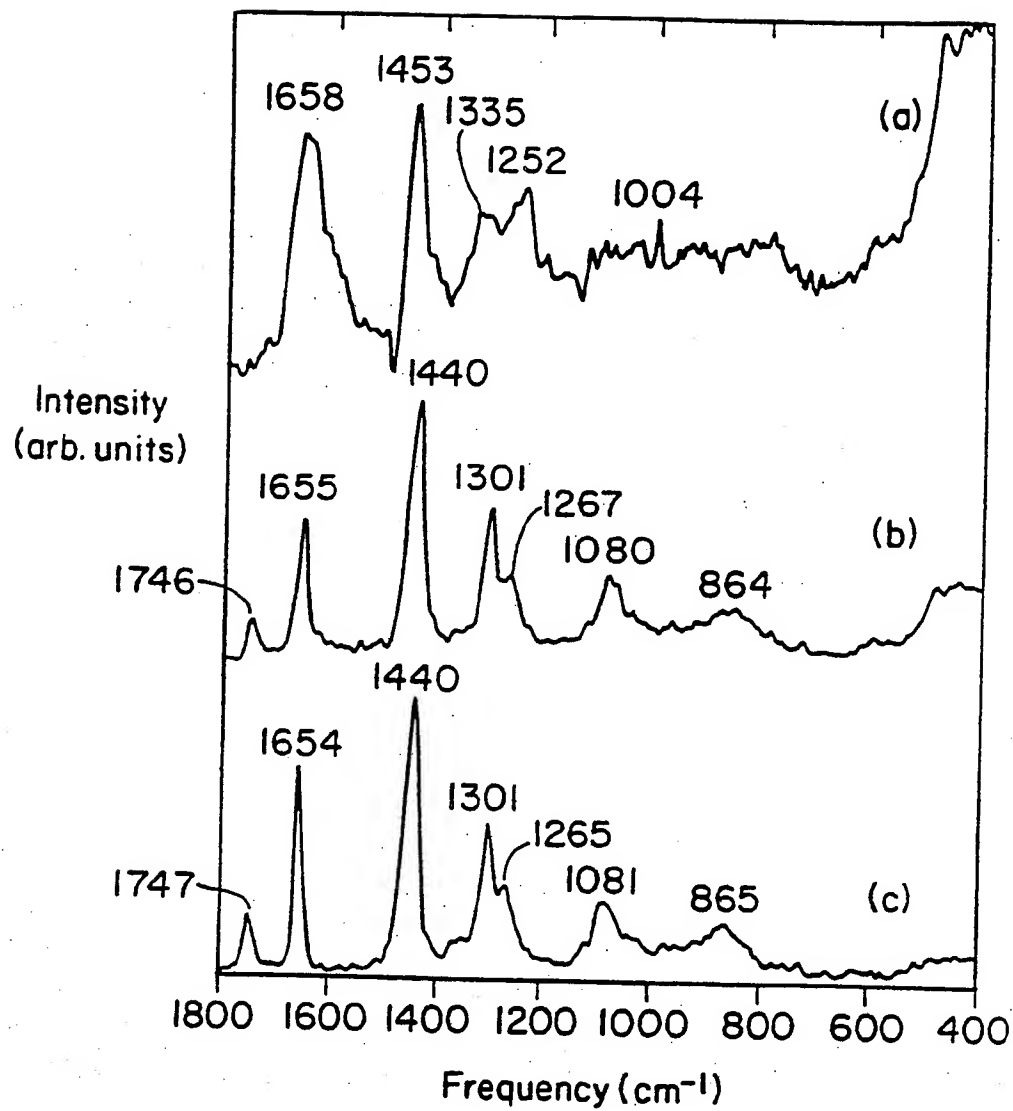


Fig. 1C

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*Fig. 3*

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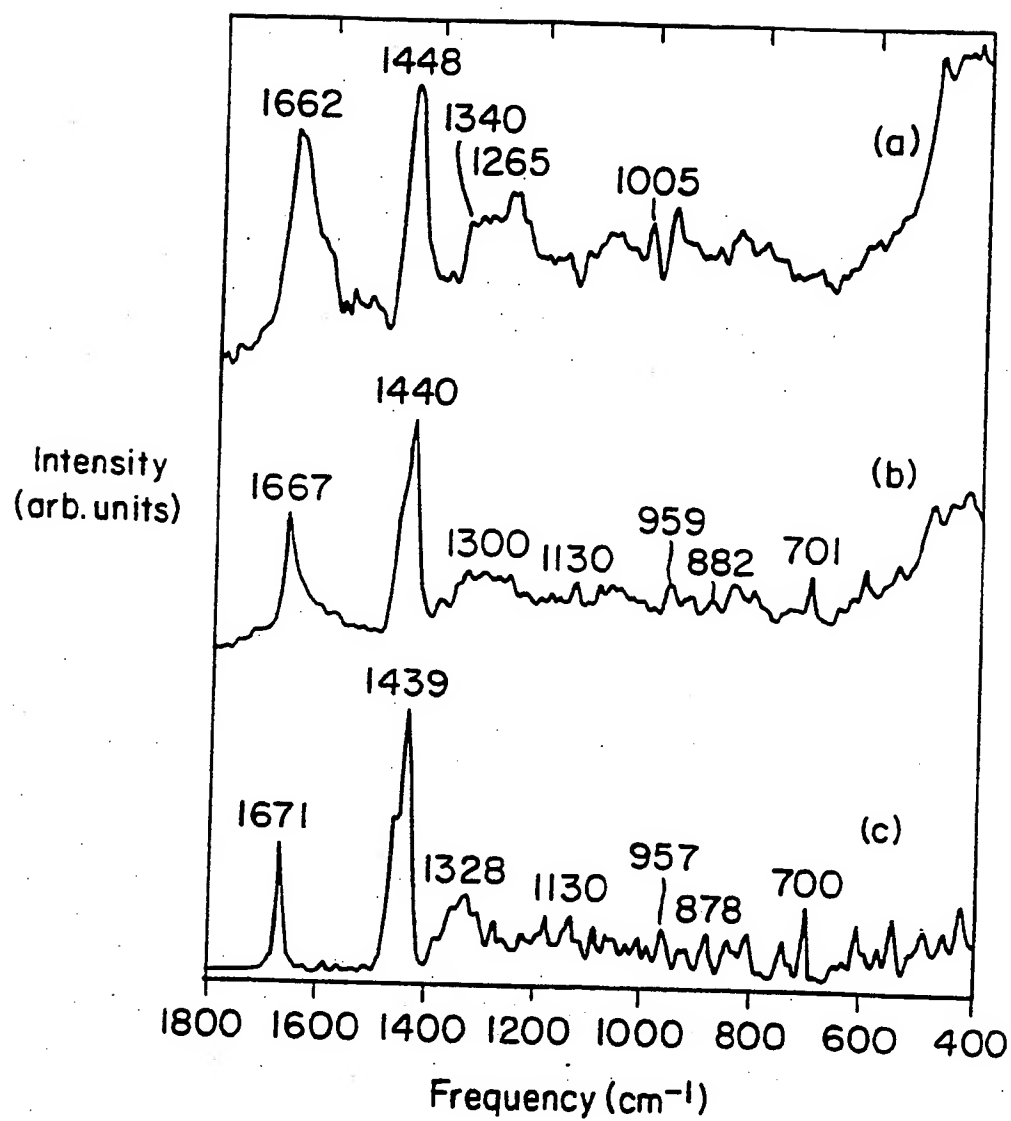
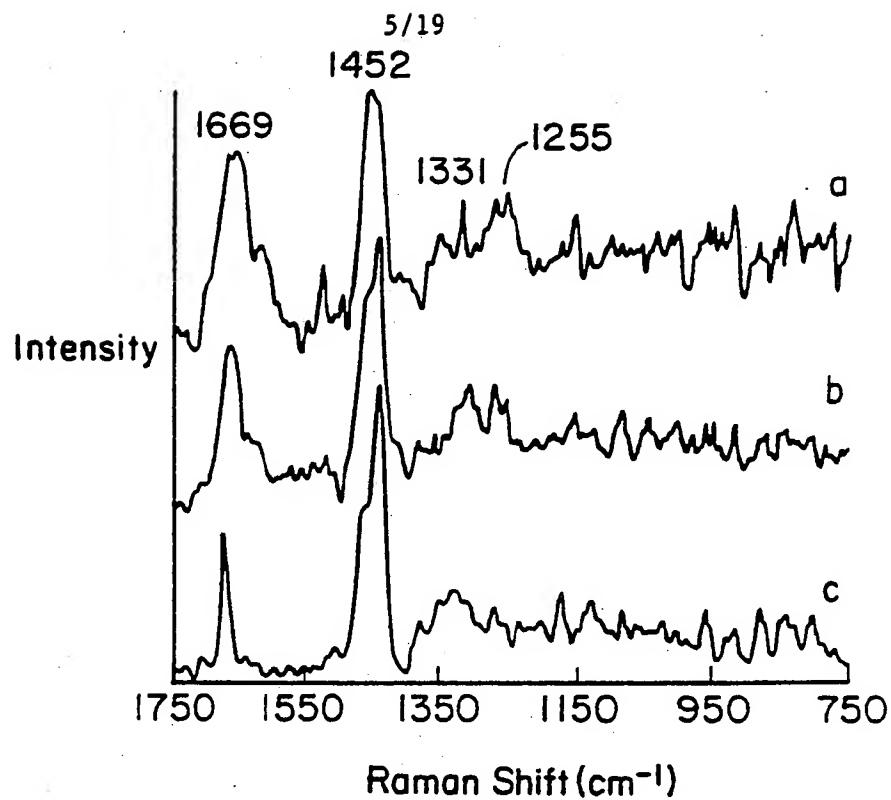
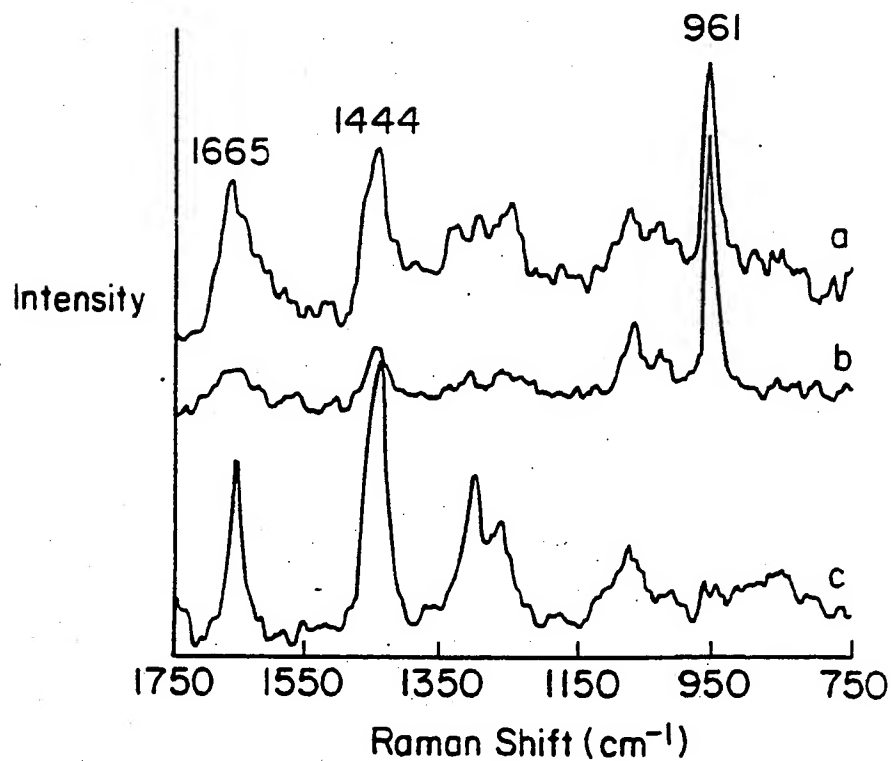
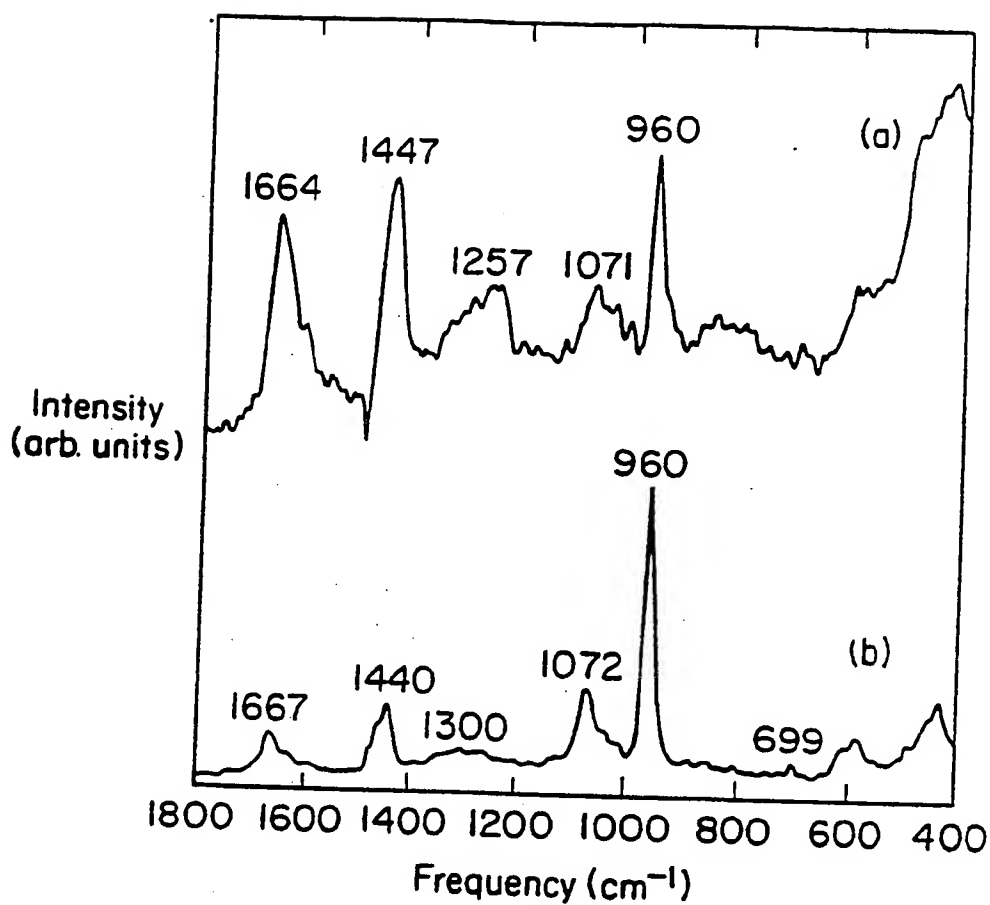
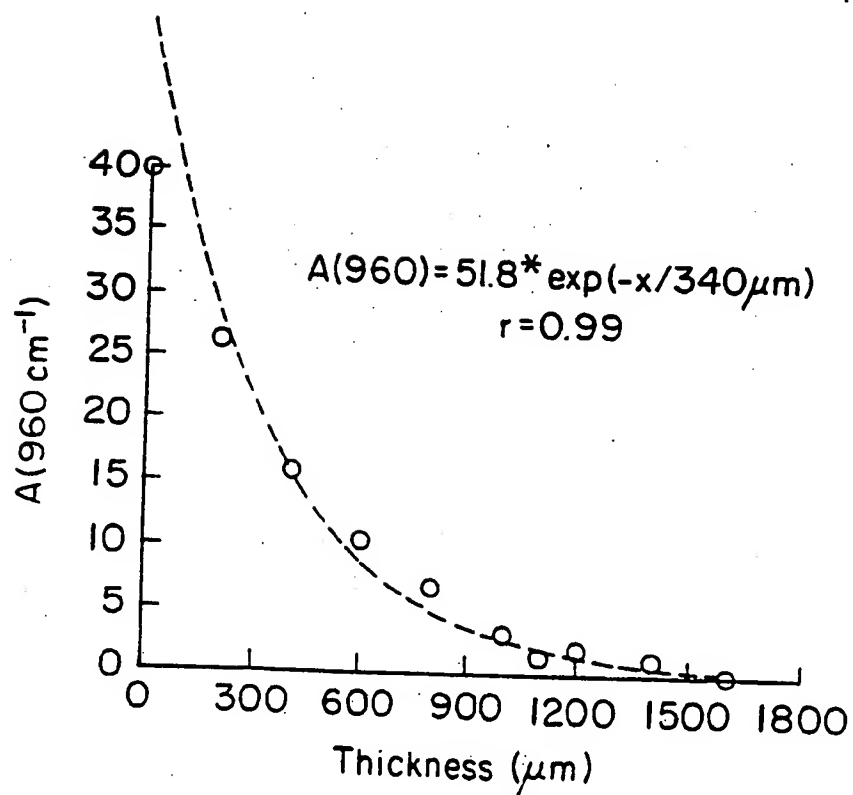


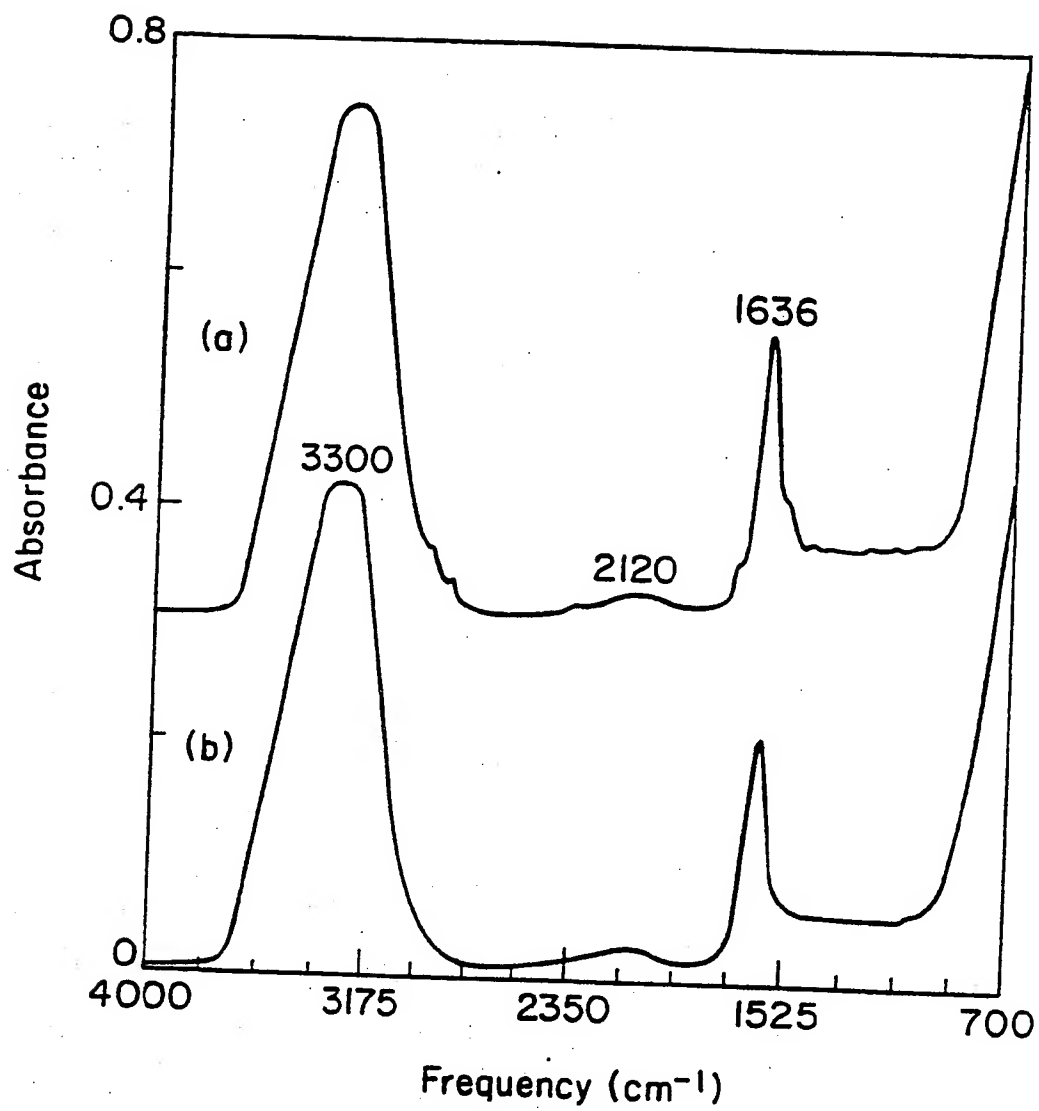
Fig. 4

*Fig. 2**Fig. 5*

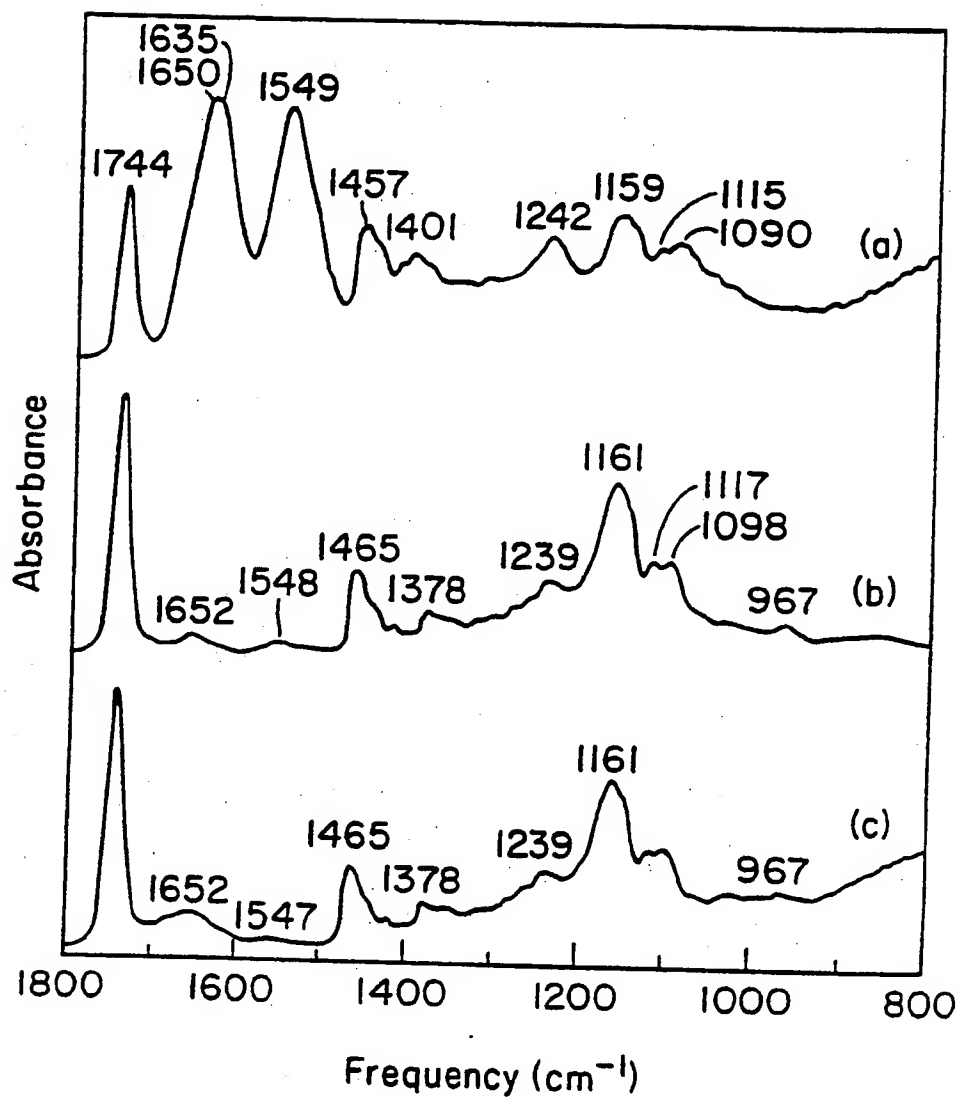
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*Fig. 6**Fig. 7*

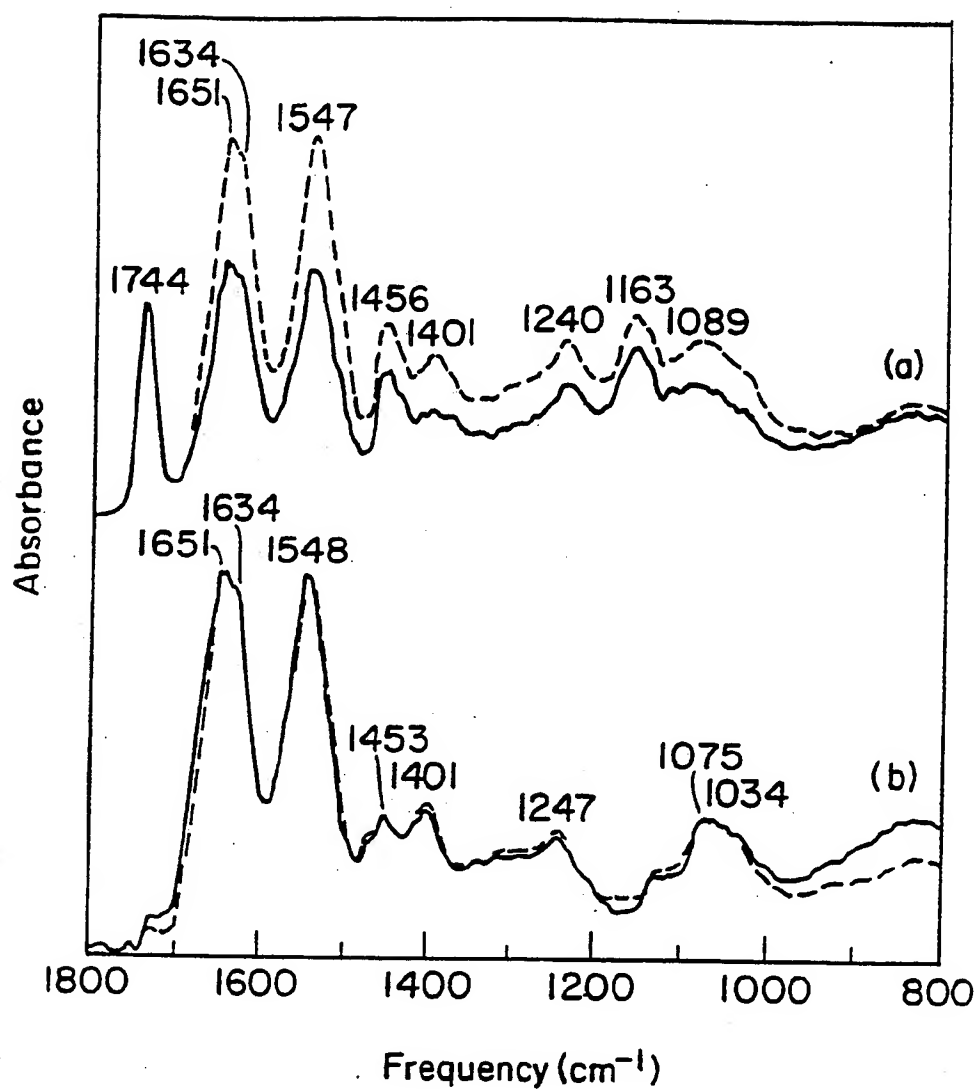
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*Fig. 8*

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*Fig. 9*

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*Fig. 10*

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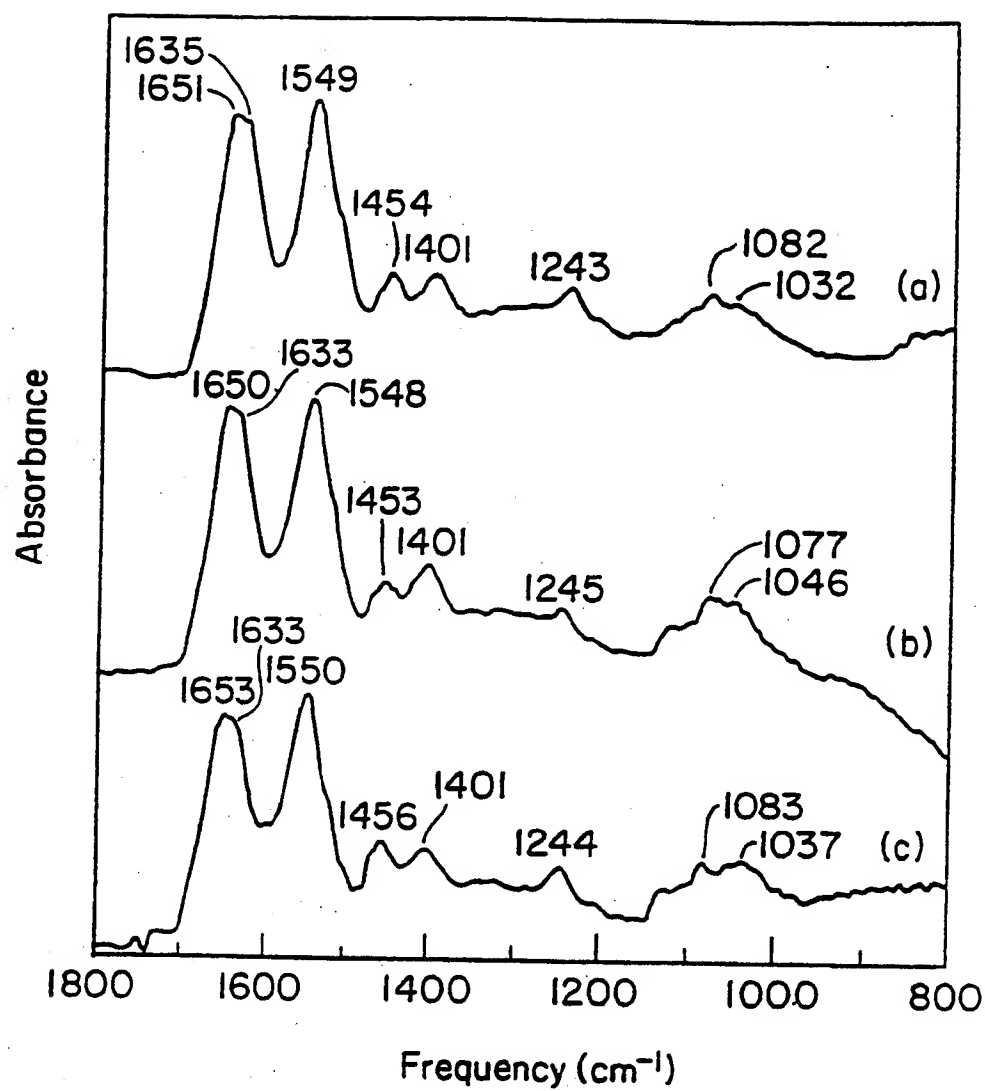
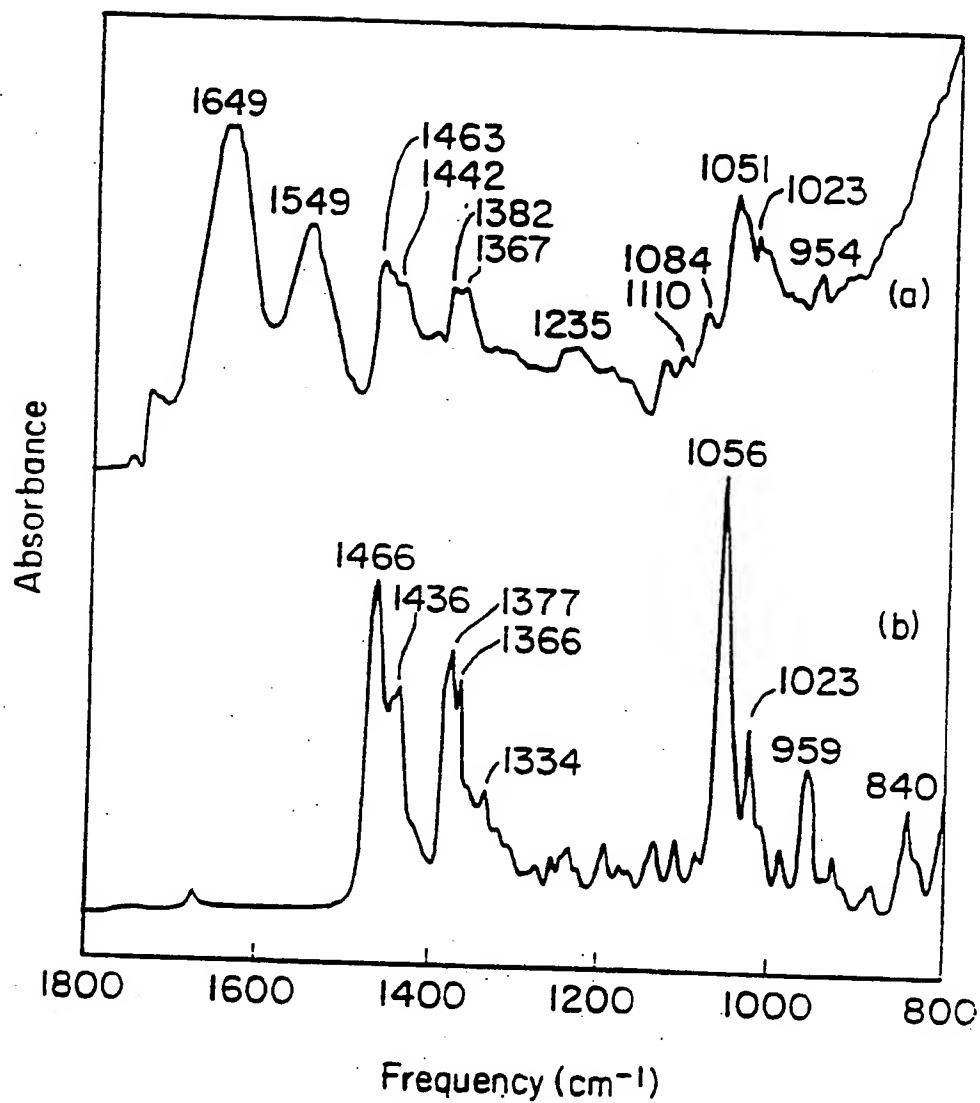
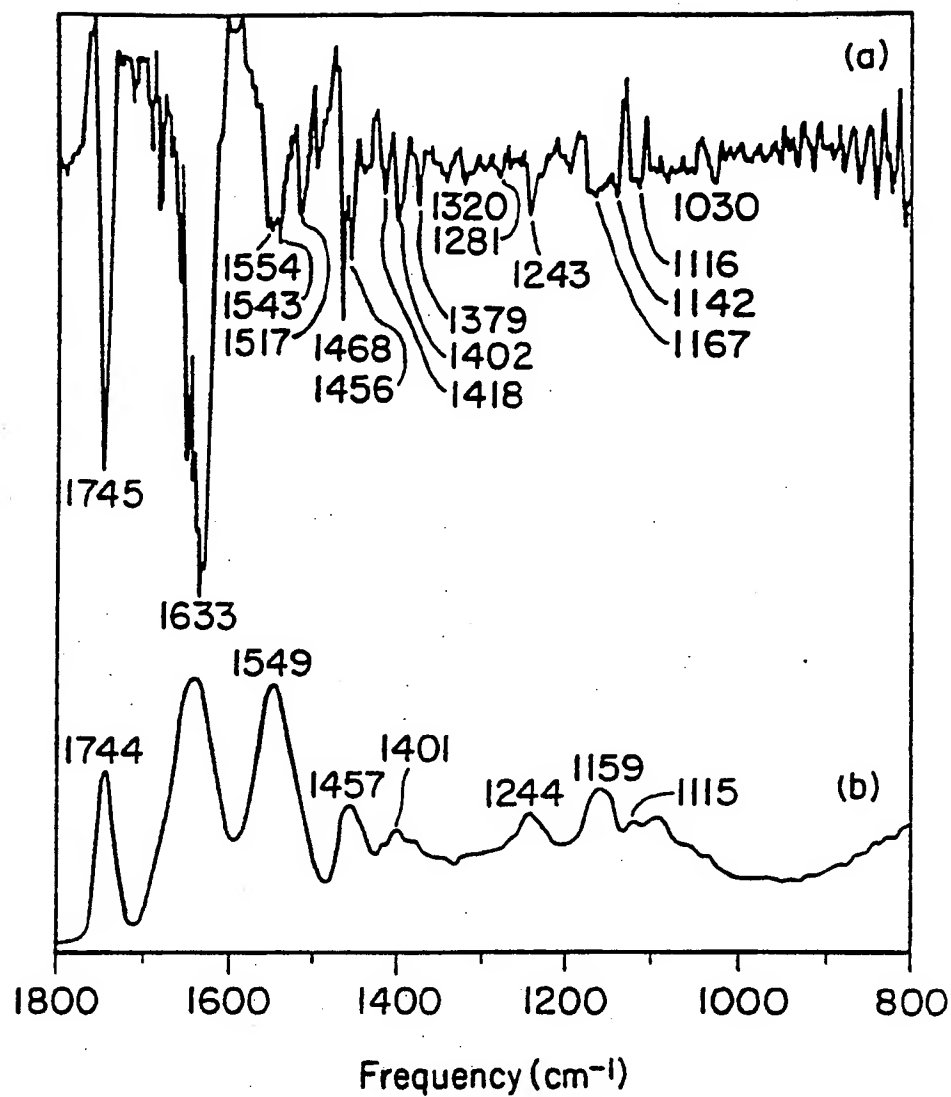


Fig. 11

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*Fig. 12*

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*Fig. 14*

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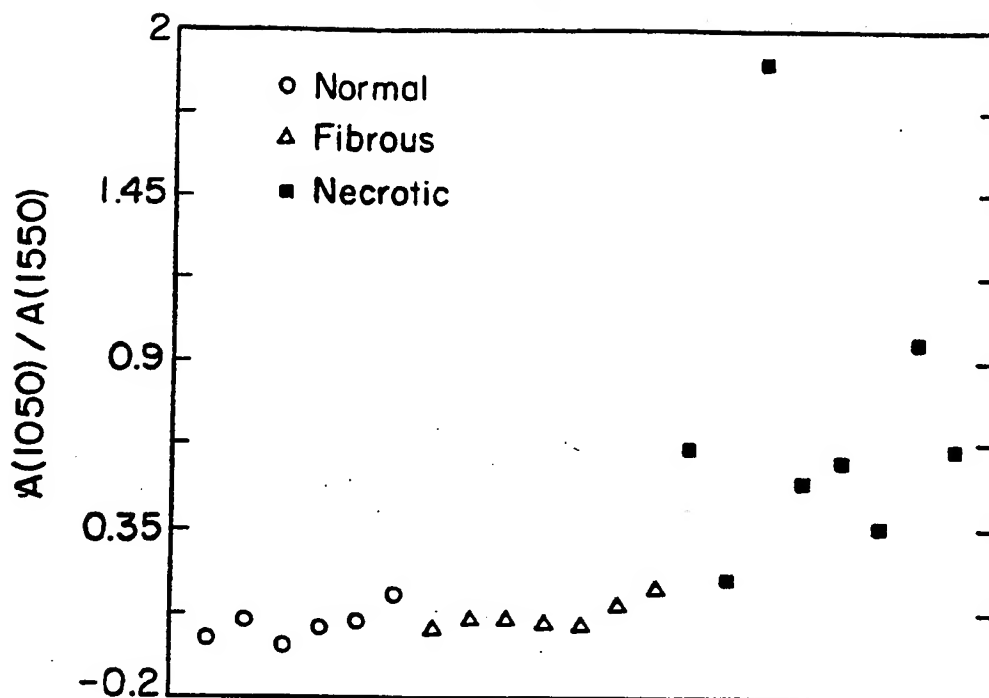


Fig. 13

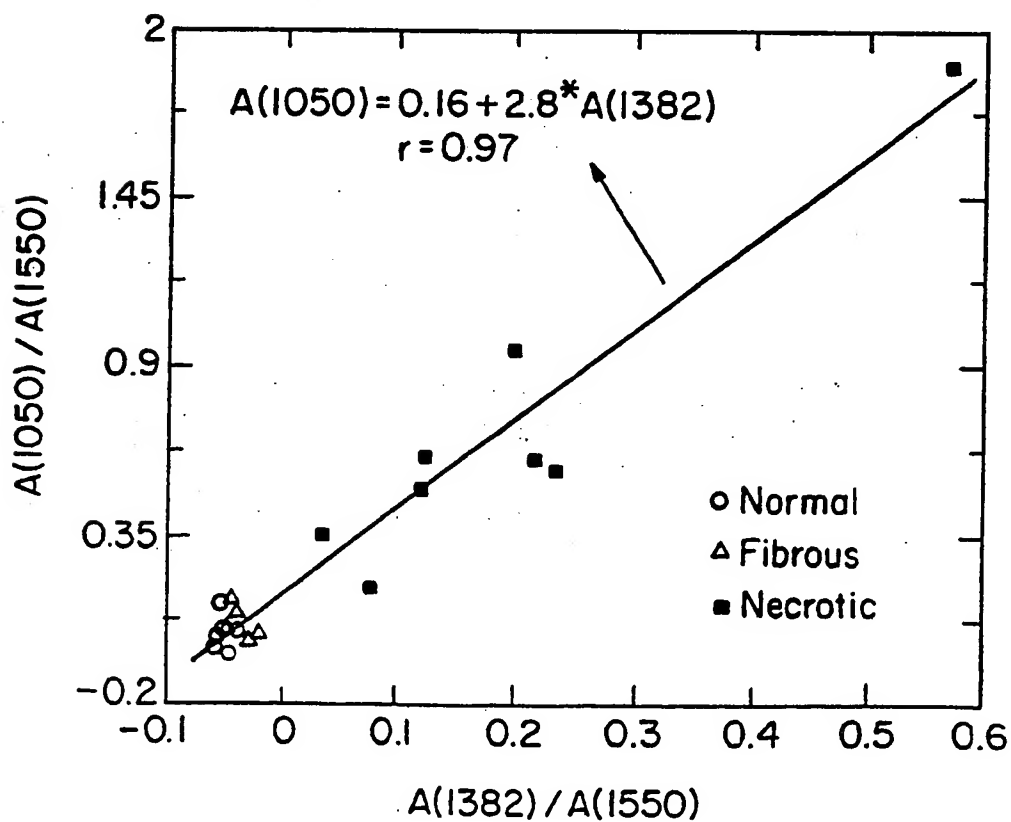
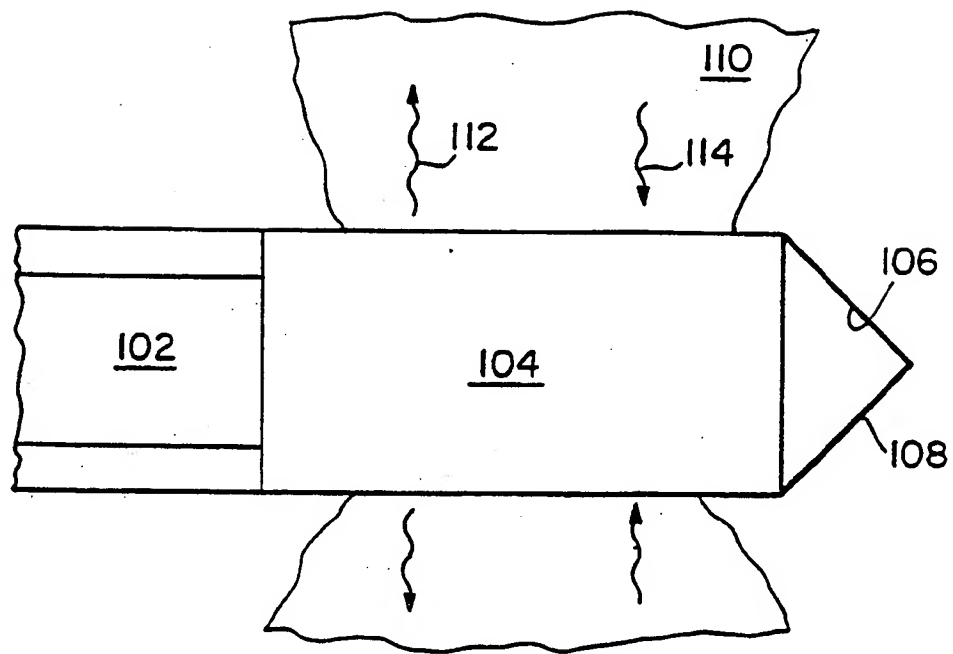
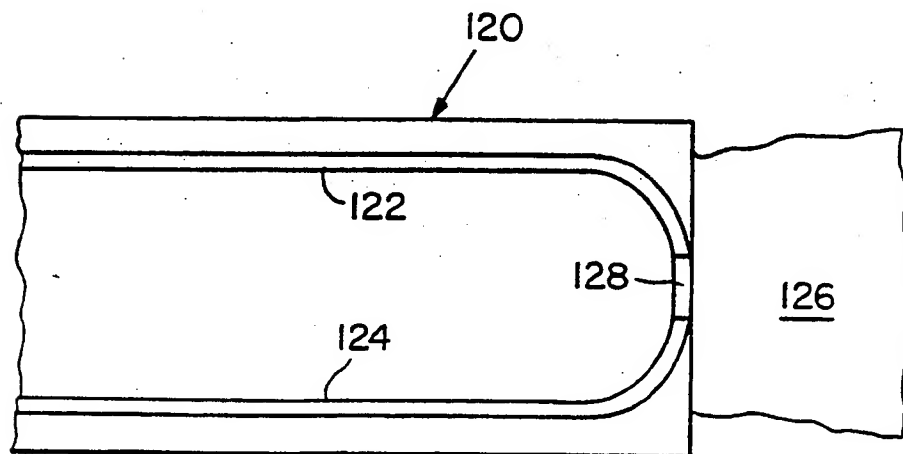


Fig. 15

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*Fig. 16A**Fig. 16B*

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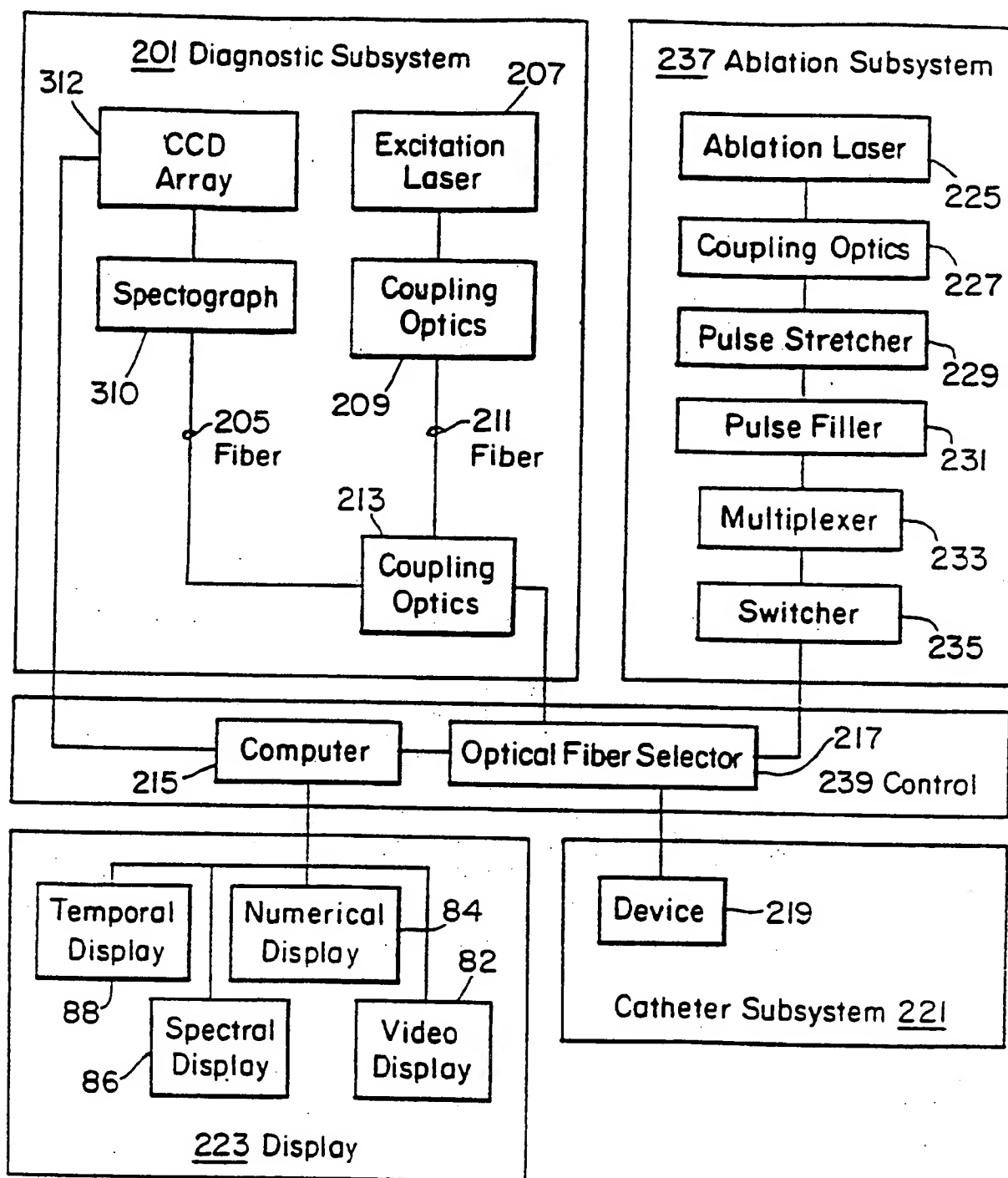


Fig. 17

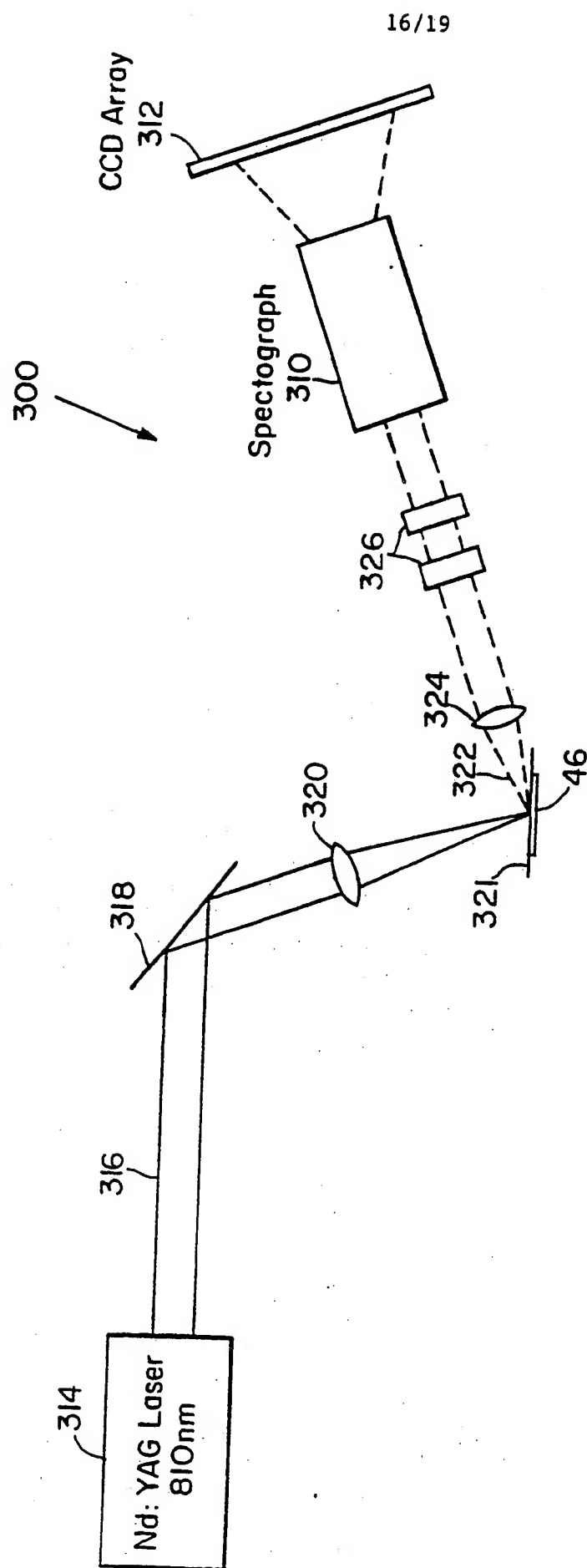
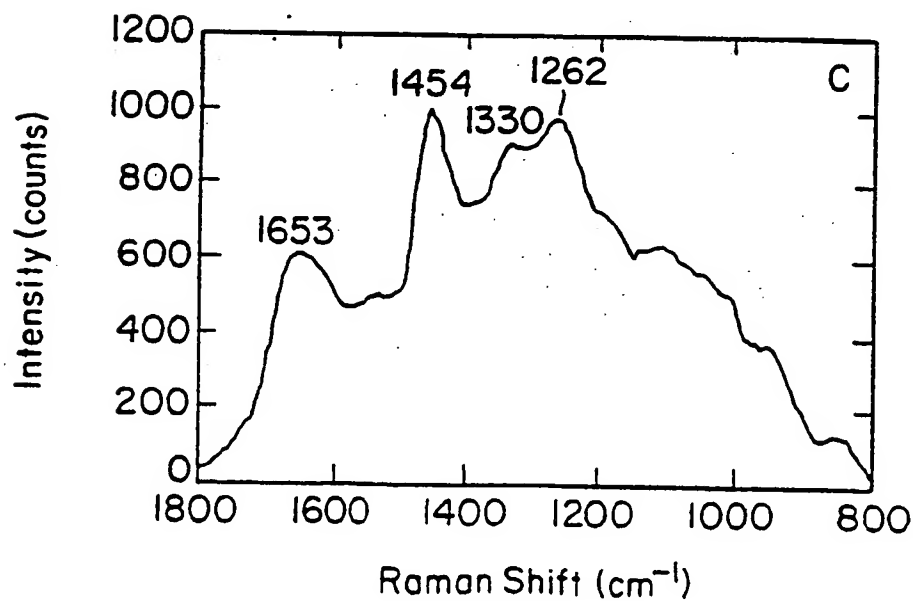
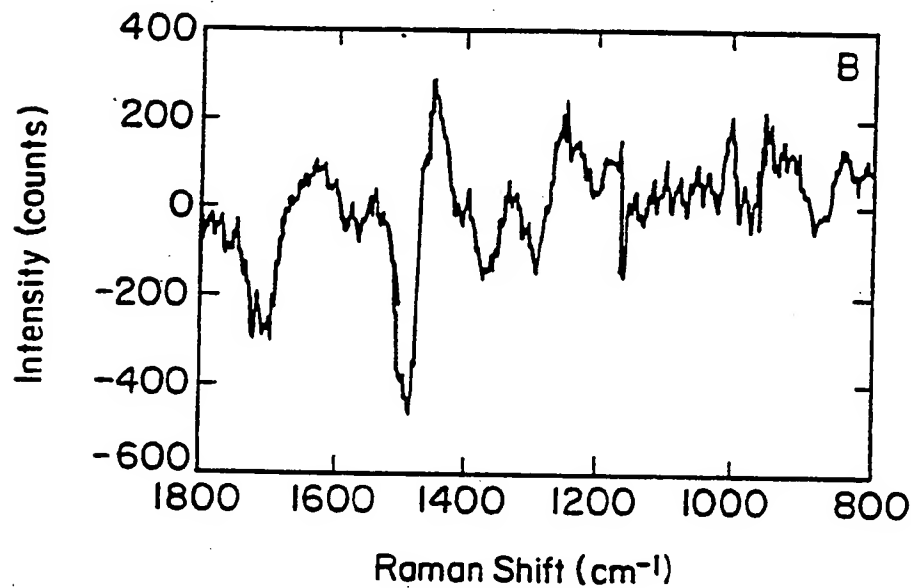
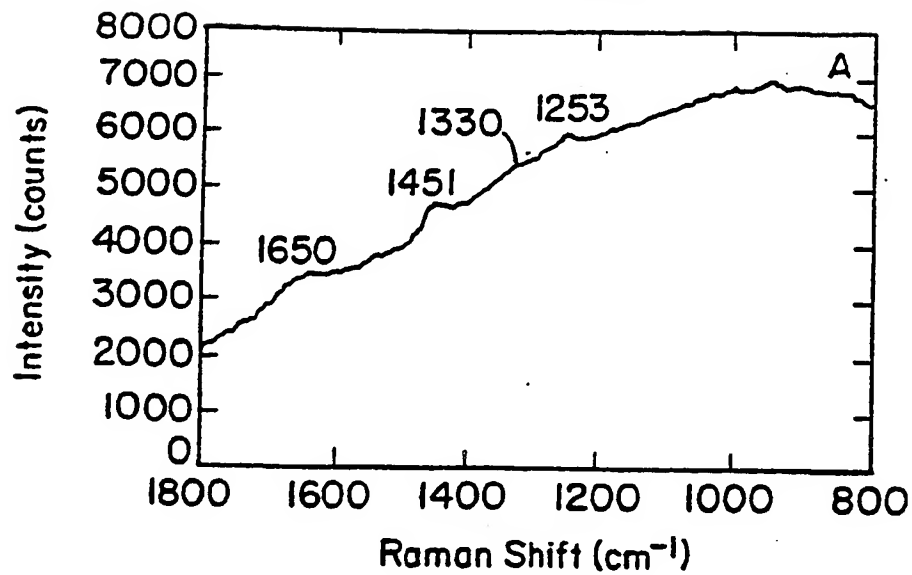


Fig. 18



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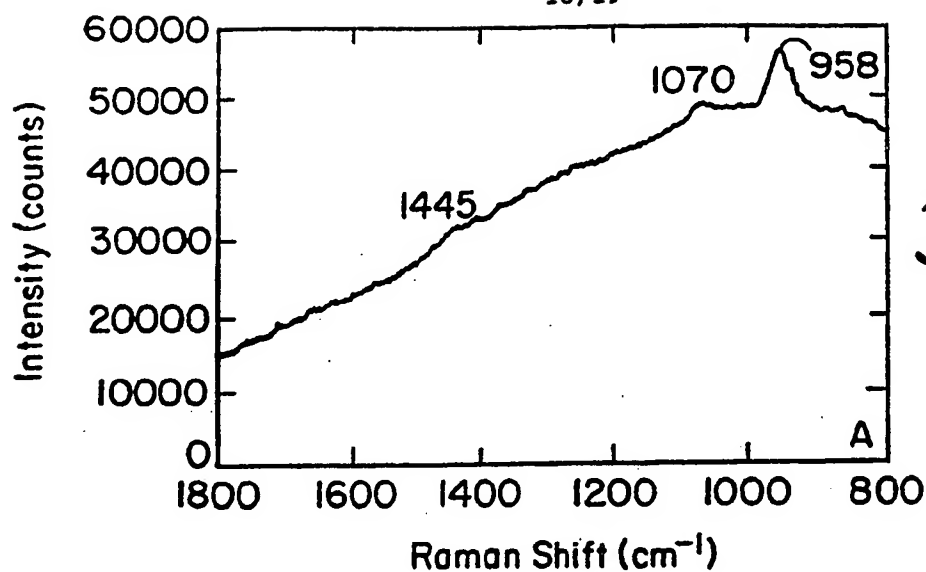


Fig. 20(A)

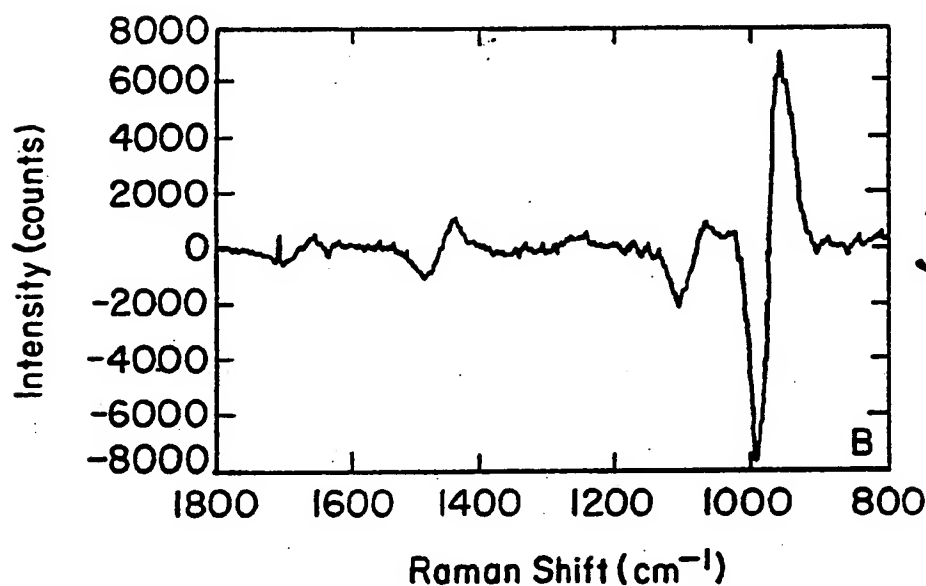


Fig. 20(B)

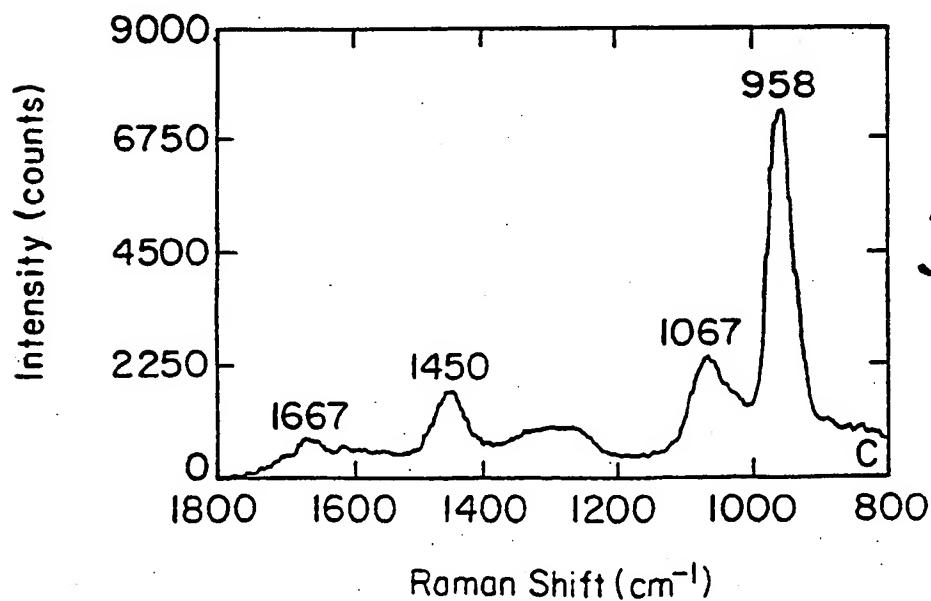


Fig. 20(C)

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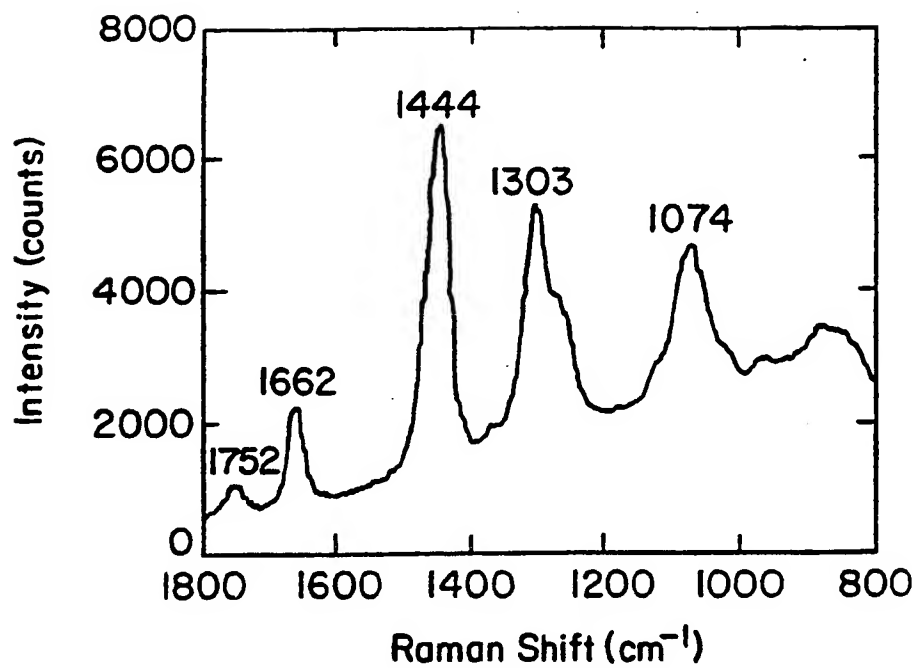



Fig. 21

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 G01N21/65; A61B5/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	G01N ; G01J ; A61B	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US,A,4 758 081 (C.BARNES) 19 July 1988 see column 3 - column 6 ---	1-8, 26-30
X	APPLIED SPECTROSCOPY vol. 43, no. 3, 1 March 1989, pages 372 - 375; J.WILLIAMSON ET AL.: 'NEAR INFRARED RAMAN SPECTROSCOPY, ETC.' see page 372 - page 375 ---	2,3,6-8, 26-30
A	APPLIED SPECTROSCOPY REVIEWS vol. 24, no. 3, 1 July 1988, pages 259 - 312; Y.OZAKI: 'MEDICAL APPLICATION OF RAMAN SPECTROSCOPY' see page 291 - page 307 ---	1,2,5,6, 26,29
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
11 JUNE 1992	22 JUN 1992	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	BOEHM C.E. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claims No.
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	
A	<p>APPLIED SPECTROSCOPY vol. 42, no. 7, 1 July 1988, pages 1188 - 1193; E.N.LEWIS ET AL.: 'DEVELOPMENT OF NEAR INFRARED FOURIER TRANSFORM RAMAN SPECTROSCOPY, ETC.' see page 1189 - page 1193 ---</p>	1-8
A	<p>ANALYTICAL CHEMISTRY vol. 56, no. 12, 1 October 1984, pages 2199 - 2204; S.SCHWAB ET AL.: 'VERSATILE EFFICIENT RAMAN SAMPLING, ETC.' see page 2199 - page 2201 ---</p>	1-4
A	<p>APPLIED OPTICS vol. 30, no. 1, 1 January 1991, pages 98 - 105; K.ONO ET AL.: 'FIBER OPTIC REFLECTANCE SPECTROPHOTOMETRY SYSTEM, ETC.' see page 99 - page 102 ---</p>	1

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9200420
SA 57003

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4758081	19-07-88	None	

(19) World Intellectual Property Organization
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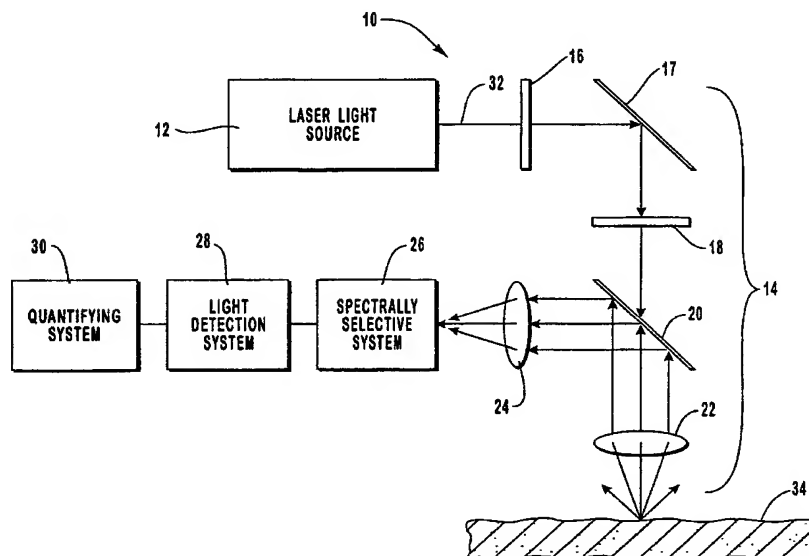
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD AND APPARATUS FOR NONINVASIVE MEASUREMENT OF CAROTENOIDS AND RELATED CHEMICAL SUBSTANCES IN BIOLOGICAL TISSUE



(57) Abstract: A method and apparatus are provided for the determination of levels of carotenoids and similar chemical compounds in biological tissue such as living skin (34). The method and apparatus provide a noninvasive, rapid, accurate, and safe determination of carotenoid levels which in turn can provide diagnostic information regarding cancer risk, or can be a marker for conditions where carotenoids or other antioxidant compounds may provide diagnostic information.

**METHOD AND APPARATUS FOR NONINVASIVE
MEASUREMENT OF CAROTENOIDS AND RELATED
CHEMICAL SUBSTANCES IN BIOLOGICAL TISSUE**

5

BACKGROUND OF THE INVENTION

1. The Field of the Invention

The present invention relates generally to techniques for measuring levels of chemical compounds found in biological tissue. More specifically, the invention relates to a method and apparatus for the noninvasive detection and measurement of levels of carotenoids and related chemical substances in biological tissue, which can be used as a diagnostic aid in assessing antioxidant status and detecting malignancy diseases or risk thereof.

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2. The Relevant Technology

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Carotenoids are plant pigments available from the diet which have important functions in the human body. The role of carotenoids in human health is a rapidly expanding area of research. Much carotenoid research has focused on their role as precursors to retinoids or vitamin A, but current research is also being conducted on other functions of carotenoids. These include antioxidant activities, modulation of the immune response, cell-to-cell communication and gap junction modulation.

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It has been demonstrated that carotenoids offer some degree of biologic protection against the formation of malignancies in various tissues. For example, carotenoids have been shown in animal models to prevent carcinoma formation in tissues such as skin, salivary gland, mammary gland, liver, and colon. In addition, low levels of carotenoids and related substances such as retinoids have been assessed as high risk factors for malignant lesions. For example, having low levels of the carotenoid lycopene has been associated with prostate and cervical cancer; the carotenoids lutein, zeaxanthin, alpha-carotene, and beta-carotene with lung cancer; and beta-carotene with oral cancer. Therefore, quantitatively measuring the chemical concentrations of these carotenoids, retinoids and other related substances provides an indicator of the risk or presence of cancer.

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The most common cancer in the United States is skin cancer. Despite attempts at patient education, skin cancer rates continue to rise. Methods to provide detection of the levels of chemicals which are associated with skin related malignancies are of great assistance to physicians and medical personnel in the early diagnosis and treatment of skin cancer.

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It has been theorized that carotenoids in the skin provide biologic protection from cutaneous malignancy. Most findings, however, have been somewhat compromised by the fact that concentrations of carotenoids in skin and skin malignancies were never measured directly, with data on levels of carotenoids in patients being derived only indirectly from blood plasma.

Prior methods used to detect the presence of chemicals associated with skin cancer have mainly been through the analysis of tissues obtained by biopsies or other invasive procedures. The standard method presently used for measuring carotenoids is through high-performance liquid chromatography (HPLC) techniques. Such techniques require that large amounts of tissue sample be removed from the patient for subsequent analysis and processing, which typically takes at least twenty four hours to complete. In the course of these types of analyses, the tissue is damaged, if not completely destroyed. Therefore, a noninvasive and more rapid technique for measurement is preferred.

A noninvasive method for the measurement of carotenoid levels in the macular tissue of the eye is described in U.S. Patent No. 5,873,831, the disclosure of which is herein incorporated by reference, in which levels of carotenoids and related substances are measured by a technique known as Raman spectroscopy. This is a technique which can identify the presence and concentration (provided proper calibration is performed) of certain chemical compounds. In this technique, nearly monochromatic light is incident upon the sample to be measured, and the inelastically scattered light which is of a different frequency than the incident light is detected and measured. The frequency shift between the incident and scattered light is known as the Raman shift, and the shift corresponds to an energy which is the "fingerprint" of the vibrational or rotational energy state of certain molecules. Typically, a molecule exhibits several characteristic Raman active vibrational or rotational energy states, and the measurement of the molecule's Raman spectrum thus provides a fingerprint of the molecule, *i.e.*, it provides a molecule-specific series of spectrally sharp vibration or rotation peaks. The intensity of the Raman scattered light corresponds directly to the concentration of the molecule(s) of interest.

One difficulty associated with Raman spectroscopy is the very low signal intensity which is inherent to Raman scattered light. It is well known that the scattered light intensity scales with the frequency raised to the fourth power. The weak Raman signal must be distinguished from Rayleigh scattered light, which is elastically scattered light of the same frequency as the incident light and which constitutes a much greater fraction of the total scattered light. The Raman signal can be separated from Rayleigh scattered light through the use of filters, gratings, or other wavelength separation devices; however, this

can have the effect of further weakening the measured Raman signal through the additional attenuation which can occur when the light passes through a wavelength separation device. In practice, the Raman scattered light is extremely difficult to detect. One might attempt to increase the Raman signal by increasing the incident laser power on the tissue sample, but this can cause burning or degradation of the sample.

In order to overcome some of these difficulties, a technique known as resonance Raman spectroscopy has been used, as described in U.S. Patent No. 5,873,831, referenced hereinabove. Such a technique is also described in U.S. Patent No. 4,832,483, the disclosure of which is herein incorporated by reference. In resonance Raman spectroscopy, the incident illumination utilized has a frequency which corresponds to the resonance frequency corresponding to electronic energy transitions of the molecules of interest. This has the effect of strongly enhancing the Raman output signal without using a higher intensity input signal, thereby avoiding damage to the sample which can be caused by laser burning. Also, these resonance Raman signals have much higher intensity than off-resonance Raman signals which are virtually invisible. Therefore, in resonance Raman spectroscopy only those Raman signals which belong to the species of interest are obtained.

In the above referenced U.S. Patent No. 5,873,831, the resonance Raman technique is used to measure the levels of the carotenoids lutein and zeaxanthin, two chemicals which are associated with healthy macular tissue of the human eye. The above referenced U.S. Patent No. 4,832,483 uses resonance Raman spectroscopy to measure certain carotenoids in blood plasma, and suggests the use of the ratios of the intensities of the Raman spectral peaks as a method of indicating the presence of various malignancy diseases.

Yet another difficulty associated with Raman measurements is that the substances of interest in the skin not only scatter incident light, but can absorb and subsequently fluoresce with substantial intensity. This fluorescence often comprises a very strong, broad signal which tends to "drown out" or overwhelm the Raman spectral peaks, thereby making identification and quantification of the substances of interest practically impossible.

Fluorescence spectroscopy is itself another technique which can be used to measure amounts of chemical compounds in biological tissue. For example, U.S. Patent No. 5,697,373 discloses use of fluorescence and/or Raman spectroscopy to detect tissue abnormality in the cervix. The disadvantage of fluorescence measurements is that since many different molecules fluoresce in broad bands of frequencies, such measurements

cannot be used to conclusively identify the presence or concentration of a particular substance.

It would therefore be a significant advance to provide a method and apparatus for the safe, noninvasive, rapid, accurate, and specific measurement of the levels of carotenoids and other similar chemical compounds which are present in varying degrees in biological tissues, and to use this information to aid in the assessment of cancer risk or disease risk in all types of biological tissue.

SUMMARY

The present invention uses the technique of resonance Raman spectroscopy to quantitatively measure the levels of carotenoids and similar substances in tissue such as skin. In this technique, monochromatic laser light is directed upon the area of tissue which is of interest. The scattered light from the tissue includes a main portion of Rayleigh scattered light, which is of the same frequency as the incident laser light. A small fraction of the scattered light is scattered inelastically at different frequencies than the incident laser light, which is the Raman signal. The Rayleigh and the Raman scattered light are separated, typically by wavelength selective filtering, and the resulting Raman signal is measured using a sensitive light detection system. The resulting Raman signal can be analyzed by a data quantifying system wherein the background fluorescence signal is subtracted and the results displayed and compared with known calibration standards.

These and other objects and features of the present invention will become more fully apparent from the following description, or may be learned by the practice of the invention as set forth hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to illustrate the manner in which the above-recited and other advantages and objects of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered limiting in scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 is a general schematic depiction of the apparatus according to the present invention;

Figure 2 is a schematic depiction of an experimental apparatus according to the present invention;

Figure 3 is a graph showing the Raman spectra obtained from living skin together with the background fluorescence spectra;

Figure 4 is a graph showing the Raman spectra of Figure 3 after the background fluorescence has been subtracted;

5 Figure 5 is a graph showing the fluorescence background of human skin measured at various laser excitation wavelengths;

Figure 6 is a graph showing the decay kinetics of human skin fluorescence;

Figure 7 is a graph showing the spectral dependencies of excitation efficiencies for Raman scattering of carotenoids and fluorescence of skin;

10 Figure 8 is a graph showing the fluorescence spectra of skin with partial bleaching of the fluorescence;

Figure 9 is a graph showing the kinetics of the bleaching behavior of human skin fluorescence;

15 Figure 10 is a graph showing the Raman spectra of living human skin measured at various locations; and

Figure 11 is a graph showing the Raman spectra of healthy skin and an adjacent carcinomic area of skin.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention is directed to a method and apparatus for the noninvasive detection and measurement of carotenoids and related chemical substances in biological tissue as well as in bodily fluids. In particular, the present method and apparatus make possible the rapid, noninvasive, and quantitative measurement of the concentration of carotenoids, as well as their isomers and metabolites, in biological tissues such as human skin. This is accomplished without the requirement of removing tissue or preparing
25 samples for HPLC analysis, as required in prior techniques.

The invention can be used in a direct and quantitative optical diagnostic technique, which uses low intensity illumination of intact tissue and provides high spatial resolution, allowing for precise quantification of the carotenoid levels in the tissue. Such a diagnostic technique can aid in the detection of tissue abnormalities such as malignancy diseases.

30 Examples of biological tissues which can be measured noninvasively with the technique of the invention include human skin, cervix, colon, and lungs. Examples of bodily fluids that can be measured include saliva, whole blood, and mucus.

The present invention employs the technique of resonance Raman spectroscopy, which is used to identify and quantify the presence of carotenoids and similar substances
35 in biological tissue such as the skin. In this technique, nearly monochromatic laser light

is directed onto the tissue and the scattered light is then spectrally filtered and detected. The scattered light comprises both Rayleigh and Raman scattered light. The Rayleigh light is light which is elastically scattered, which means it is scattered at the same wavelength as the incident laser light. Most of the scattered light is scattered elastically.

5 A small remainder of the light is scattered in an inelastic fashion, and is therefore of different frequencies than the incident laser light. This inelastically scattered light forms the Raman signal. The frequency difference between the laser light and the Raman scattered light is known as the Raman shift and is typically measured as a difference in wave numbers (or difference in frequencies or wavelengths). The magnitude of the

10 Raman shifts is an indication of the type of chemical present, and the intensities of the Raman signal peaks correspond directly to the chemical concentration. One of the reasons why Raman spectroscopy is so useful is that specific wave number shifts correspond to certain modes of vibrational or rotational eigenstates associated with specific chemical structures, and hence provide a "fingerprint" of these chemical

15 structures. The Raman shift is independent of the wavelength of incident light used, and hence, in theory, any strong and fairly monochromatic light source can be used in this technique.

The technique of resonance Raman spectroscopy used in the present invention aids in overcoming the difficulties associated with measuring the inherently weak Raman signal. In resonance Raman spectroscopy, a laser source of wavelength near the

20 absorption peaks corresponding to electronic transitions of the molecules of interest is utilized. By making the incident light close to resonant with the electronic absorption frequencies of the molecules of interest, the Raman signal is substantially enhanced, which provides the advantage of being able to use lower incident laser power (which in turn

25 minimizes tissue damage) and also results in less stringent requirements for the sensitivity of the detection equipment.

Raman spectroscopy in tissues using short visible laser wavelengths normally would be impossible due to high native fluorescence, particularly in skin tissue, which masks the weaker Raman signals. The present invention uses short visible wavelengths

30 in a way which selectively and drastically increases the carotenoid Raman signal due to selective resonance coupling of the laser with this family of molecules, which themselves are known to exhibit only very weak fluorescence. This signal enhancement allows carotenoid levels to be determined even in the presence of the strong native fluorescence. Since the tissue not only scatters the light (elastically and inelastically), but also absorbs

the light, background fluorescence is produced during Raman spectroscopy measurements. As discussed in further detail below, the background fluorescence can be subtracted from the Raman spectrum and the resulting spectrum can be expanded to give a clear indication of the Raman carotenoid signals. It is unexpected that a useful Raman signal can be measured at the low laser power levels in the visible wavelength range utilized in the present invention as normally the Raman signal would be buried in the typically high fluorescence background of human tissue.

In a method for the noninvasive measurement of carotenoids and related chemical substances in biological tissue according to the present invention, a light source such as a laser is utilized which generates light at a wavelength that produces a Raman response with a wavelength shift for the carotenoids to be detected. The laser light is directed onto the tissue, with the light having an intensity which does not cause destruction of the tissue and does not substantially alter carotenoid levels. The elastically and inelastically scattered light from the tissue is collected, with the inelastically scattered light having characteristic energy shifts and quantifiable intensities which produce a Raman signal corresponding to carotenoids in the tissue. The elastically scattered light is filtered, and the intensity of the inelastically scattered light forming the Raman signal is quantified.

The intensity of the light scattered inelastically from the carotenoid molecules and forming the Raman signal can be compared with the intensity of Raman scattering from normal biological tissue to assess the risk or presence of a malignancy disease such as cancer in a live subject. For example, a substantial difference between the intensity of the Raman signal of suspected malignant biological tissue and the intensity of Raman scattering from adjacent normal biological tissue indicates the presence or risk of disease. The intensity of the Raman signal can also be quantified to assess the antioxidant status of the tissue.

Figure 1 is a general schematic depiction of the apparatus of the present invention, generally labeled 10, for measuring carotenoids and like substances in biological tissue using Raman spectroscopy. The apparatus 10 includes a coherent light source 12, which in one preferred embodiment is a low power argon ion laser. Alternatively, light source 12 may comprise other devices for generating nearly monochromatic light. The light source 12 generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected. Preferably, in the case of carotenoids, light source 12 generates laser light in a range from about 450 nm to about 520 nm, which corresponds to the absorption band of the carotenoids of interest. Such laser light is readily available from commercially produced argon lasers. For example, blue/green argon laser lines can

be used to resonantly excite the electronic absorption of the carotenoids, such as the 4880 Å or 5145 Å lines of an argon laser. It should be understood, however, that the present invention is not limited to light generated within these wavelengths, since other wavelengths of light could be used if desired, *e.g.*, UV laser lines overlapping the absorption transitions of carotenoids occurring in the ultraviolet spectral region.

The light source 12 is in optical communication with a light beam delivery and collection system 14 which can include various optical components for directing laser light to the tissue to be measured and collecting the scattered light. As shown in Figure 1, the optical components of delivery and collection system 14 include a neutral density filter 16, a diffraction grating 17, a slit 18, a beam splitter 20, a first lens 22, and a second lens 24. The interaction of these optical components with a laser beam from light source 12 will be discussed in further detail below.

The delivery and collection system 14 is in optical communication with a spectrally selective system 26 such as a Raman spectrometer, which performs the function of spectral separation of the Raman scattered light from Rayleigh scattered light. The spectrally selective system 26 can include various optical components such as grating monochromators, holographic filters, dielectric filters, acousto-optic filters, prisms, combinations thereof, and the like.

The spectrally selective system 26 is in optical communication with a detection means such as a light detection system 28, which is capable of measuring the intensity of the Raman scattered light as a function of frequency in the frequency range of interest such as the frequencies characteristic of carotenoids in the skin. The light detection system 28 may comprise, but is not limited to, devices such as a CCD (Charge Coupled Device) detector array, an intensified CCD detector array, a photomultiplier apparatus, photodiodes, or the like.

The spectrally selective system 26 and light detection system 28 can be selected from commercial spectrometer systems such as a medium-resolution grating spectrometer employing rapid detection with a cooled charge-coupled silicon detector array. For example, a monochromator can be used which employs a dispersion grating with 1200 lines/mm, and a liquid nitrogen cooled silicon CCD detector array with a 25 µm pixel width. Another suitable spectrometer is a holographic imaging spectrometer, which is interfaced with a CCD camera and employs a volume holographic transmission grating. The spectrally selective system 26 and light detection system 28 can also be combined into a Raman imaging system that includes spectrally selective optical elements used in association with a low light level CCD imaging array such as an intensified CCD camera.

The detected light is preferably converted by light detection system 28 into a signal which can be visually displayed on an output display such as a computer monitor or the like. It should be understood that the light detection system 28 may also convert the light signal into other digital or numerical formats, if desired. The resultant Raman signal intensities are preferably analyzed via a quantifying means such as a quantifying system 30, which may be calibrated by comparison with chemically measured carotenoid levels from other experiments. The quantifying system 30 may be a computer, preferably one in which data acquisition software is installed that is capable of spectral manipulations, such as subtraction of the background fluorescence spectrum, thereby allowing for a background-free Raman signal while using safe laser power densities. The quantifying system 30 may also comprise a CCD image display or monitor. The quantifying system 30 may be combined with the output display in one computer and can calibrate the results with carotenoid levels obtained from other experiments such that the signal intensity is calibrated with actual carotenoid levels.

During operation of apparatus 10, a laser beam 32 is generated from light source 12 and is directed through an input optical fiber to delivery and collection system 14. The laser beam 32 is directed through neutral density filter 16 which reduces the laser power, and is reflected off of diffraction grating 17 and passed through slit 18 to eliminate laser plasma lines. The beam is then directed through beam splitter 20 and weakly focused by first lens 22 onto a tissue 34 to be measured. The power density or light intensity of the beam is preferably in a range up to about 200 mW/cm² at an exposure time of about 1 ms to about 10,000 s. The backscattered light from tissue 34 is then collected by first lens 22 and reflected off of beam splitter 20 toward second lens 24, which focuses the light into an output optical fiber for routing the light to spectrally selective system 26 such as a Raman spectrometer. After the Raman signal has been separated from the Rayleigh light in spectrally selective system 26, the Raman signal is directed to light detection system 28, which measures the light intensity as a function of frequency in the range covering the Raman peaks of interest, approximately 800 to 2000 cm⁻¹ for carotenoids. The light detection system 28 then converts the Raman signal into a form suitable for visual display such as on a computer monitor or the like, and the resultant Raman signal is analyzed via quantifying system 30.

The present invention is particularly useful in the detection of total carotenoid content in human skin. Several of the carotenoids which have been found to be associated with healthy skin include all-*trans*- β -carotene, lycopene, α -carotene, γ -carotene, phytoene, phytofluene, septapreno- β -carotene, 7,7' dihydro- β -carotene, astaxanthin,

canthaxanthin, zeaxanthin, lutein, β -apo-8'-carotenal, violaxanthin, and rhodoxanthin. These are chain-like molecules with different lengths and attachments, all having a carbon backbone with alternating carbon double and single bonds, respectively. The vibration of these bonds, common to all carotenoids, can be detected with Raman spectroscopy. It is known from separate measurements that the wavenumber shifts of these carotenoids are generally in the range from 800 to 2000 cm^{-1} (wavenumbers). For example, the carotenoids lutein and zeaxanthin are known to have wavenumber shifts of approximately 1160 cm^{-1} and 1520 cm^{-1} , respectively.

Carotenoids are an important component of the skin's antioxidant defense systems, where they are thought to act as free radical and singlet oxygen scavengers. Furthermore, carotenoids protect the skin from a number of harmful reactive oxygen species (ROS), which are formed, for example, by excessive exposure of skin to ultra-violet (UV) light such as from sunlight. The ROS can potentially cause oxidative cell damage and the formation of skin cancers such as basal cell carcinoma, squamous cell carcinoma, and malignant melanoma. In addition, UV light exposure can lead to immuno-suppression and premature skin aging. Once formed, the ROS efficiently react with DNA, proteins, and unsaturated fatty acids, causing DNA strand breaks and oxidative damage, as well as protein-protein and protein-DNA cross links. Oxidation of lipids can result in the formation of lipid peroxides which persist a relatively long time in the cells and can thus initiate radical chain reactions and enhance oxidation damage.

It has been previously demonstrated that there is a correlation between the levels of carotenoids, retinoids, and similar chemical substances in the skin and the risk of skin cancer and other skin disorders. People with low levels of carotenoids in their skin are at a significantly greater risk of getting skin cancer. Therefore, if a determination can be made of the levels of carotenoids which are present in the skin, the risk for cancer can be assessed; and if low levels of carotenoids are measured, preventative steps can be taken, such as dietary supplements.

Current methods for evaluating the presence of skin cancer generally include excising an area of the suspected tissue and performing a histological analysis. This is an invasive procedure and is usually performed in the later stage of cancer, and thus is not useful in early detection of cancer or precancerous conditions in an efficient and timely manner in order to provide proper treatment. The present invention overcomes these difficulties by providing for early noninvasive measurement of carotenoids to aid in the determination of cancer risk.

The present invention not only provides for a rapid, non-invasive assessment of

carotenoid levels in a variety of human tissues and bodily fluids, but also has many additional beneficial uses. These include assessing the overall antioxidant status in human tissue; providing for early cancer detection using spatially resolved Raman data or Raman images; providing a screening tool suitable for use in large population studies of cancer prevention and other diseases involving carotenoids or other antioxidants; providing for monitoring of dietary manipulation of tissue carotenoid or other antioxidant content; and providing a tool to assess carotenoid distribution and uptake from cosmetic compounds.

The methods and apparatus of the invention are especially effective in measuring the carotenoid levels in skin, skin lesions, and skin malignancies. The present invention allows two-dimensional Raman mapping to be developed which will provide a non-invasive method for defining tumor margins, thus eliminating time consuming and tedious sections and allowing for instant intraoperative tumor margin delineation. The measurement of carotenoid levels can also be used as a predictor of malignant potential of individual cutaneous lesions.

Various experiments were performed which demonstrate that strong Raman signals are readily obtainable for various areas of living human skin using low light exposures. The following examples set forth the apparatus and procedures utilized in these experiments as well as the results derived therefrom.

Example 1

An experimental apparatus 40 suitable for Raman measurements of carotenoids in human skin was assembled as shown schematically in Figure 2. The apparatus 40 includes a light module 42 which contains the light beam delivery and collection optics, and a Raman module 44 which contains the spectrometer components. The light module 42 is designed as a hand-held beam delivery and collection device, and can be placed in close proximity to the scattering sample (*e.g.*, human skin) such that the sample is within about 5 cm of the light collecting optics. This results in an apparatus with a high f-number and hence high light throughput.

As excitation source, the blue/green lines of an argon laser 46 were used. The argon laser 46 is in optical communication with light module 42 such that the excitation laser light is routed through an input optical fiber into light module 42 during operation. A portion of the laser light is split off by a beam splitter and sampled with a photodetector for reference purposes (not shown) prior to entering light module 42. The laser light is coupled out of the input optical fiber and into light module 42 where it is collimated with a first lens 48 and passed through a first narrow bandwidth filter 50 (*e.g.*, dielectric interference filter or holographic notch filter). The light is then reflected off of a pair of

dichroic beam splitters 52 and 54, and is directed onto living skin tissue 36 via a second lens 56. The narrow bandwidth filter 50 serves to remove laser plasma lines, potential fiber emission and fiber Raman scattering. The beam splitters 52 and 54 are dielectric and coated to pass wavelengths of 488 nm and simultaneously reflect wavelengths larger than about 500 nm. The laser spot size on the skin tissue can be manipulated by proper choice of the focal length of lens 56. In the present experiments, a skin spot size of about 2 mm was illuminated with laser light of about 200 mW/cm² power density (which is considered safe by ANSI standards).

The Raman shifted signals from the skin tissue are collected in a 180 degree backscattering geometry. The scattered light is collected and collimated by lens 56 and routed towards an output optical fiber leading to Raman module 44, via beam splitter 54, a second narrow bandwidth filter 58, and a third lens 60. The filter 58 is designed to reject the Rayleigh component of the Raman scattered light and to simultaneously transmit the carotenoid Stokes signals with high light throughput.

The Raman module 44 is a commercially available grating spectrometer, and is interfaced to a CCD camera 62 with a silicon detector array. The Raman scattered light from light module 42 is coupled out of the output optical fiber and sent to a reflection grating 64 via a first mirror 66. The light is reflected from reflection grating 64 as wavelength dispersed signals and imaged onto the detector array of CCD camera 62 via a second mirror 68. Besides employing a single grating stage for light dispersion and thus allowing for high light throughput, the Raman module 44 is the size of a shoe-box and therefore compact, movable and suitable for use on human subjects. The CCD camera 62 is operatively connected with a personal computer 70 such that the signals imaged on the detector array are displayed on a monitor of computer 70.

Example 2

A typical Raman carotenoid spectrum obtained from the skin of a healthy human volunteer with the apparatus of Example 1 is shown in the graph of Figure 3. A skin spot size of about 2 mm was illuminated with laser light at 488 nm with 10 mW power. The data was plotted in the standard format of photon count (intensity) vs. wavenumber shift. The spectrum was measured using resonance Raman techniques for further enhancement of the inherently weak Raman signal. Raman peaks characteristic of carotenoid molecules appear in the graph of Figure 3, which are superimposed on a broad fluorescence background. Nevertheless, the Raman peaks are clearly resolved, and, using the high dynamic sensitivity range of the CCD detector, can be displayed with good sensitivity resolution and high signal-to-noise ratio. This is shown for example in Figure 4, where

the fluorescence background has been fitted with a higher order polynomial and subtracted from the spectrum. The background fluorescence spectrum can be subtracted by commercially available spectral acquisition software (*e.g.*, Kestrel Spec, available from Rhea Corp). The two peaks correspond to the 1159 and 1524 cm^{-1} carbon-carbon single and double bond stretching vibrations, respectively, of the carotenoid molecules, and their peak heights correlate with existing carotenoid concentrations in the skin.

Example 3

In order to further characterize the fluorescence background and its influence on the Raman measurements of human skin, the fluorescence emission spectra of human skin was measured *in vivo* for blue/green laser excitation wavelengths of 458 nm, 488 nm, 514.5 nm, and 532 nm. The power density of the laser was 0.2 W/cm^2 , and the sampling time per 1 nm wavelength interval was 1 second. The results are shown in the graph of Figure 5, revealing that the emission consists of at least two broad and overlapping bands, one centered near 600 nm, and the other near 750 nm. With increasing excitation wavelengths, the emission central maxima shift slightly to longer wavelengths, their overall intensities decrease, and at 532 nm excitation only the short wavelength component of the emission is left. The origin of this so called skin "autofluorescence" is due to intrinsic fluorophores, *i.e.* collagen cells, porphyrin molecules, etc., and not due to carotenoid emission. This conclusion is further supported by the decay kinetics of the fluorescence, shown in the graph of Figure 6. This graph plots the fluorescence intensity at ~600 nm as a function of time after excitation with short (100 ps) pulses from a mode-locked laser at 532 nm. Plotted on a semi-logarithmic scale, the intensity is seen to decay almost singly-exponentially with a lifetime of ~6 ns. This value is typical for the spontaneous emission lifetime of native fluorophores present in the skin, and is orders of magnitude larger than the lifetime reported for carotenoids (~200 fs).

Example 4

The Raman scattering intensity scales inversely with the fourth power of the excitation wavelength, which in practice, means that it strongly increases with shorter excitation wavelength. On the other hand, the overlapping fluorescence which masks the Raman signal, also increases with shorter wavelength. In the case of resonance Raman scattering, the light scattering efficiency is additionally influenced by the electronic absorption behavior of the scattering species, following in general the spectral dependence of the electronic absorption transition. Therefore, in order to find the optimum excitation conditions for maximum contrast between Raman peaks and fluorescence background, the excitation conditions were varied using five different argon laser lines and a frequency

doubled Nd:YAG laser wavelength. The excitation power density was 0.2 W/cm^2 , and the sampling time was 10 seconds. The results are given in the graph of Figure 7, which shows the spectral dependencies for both the strongest Raman carotenoid peak intensity at 1520 cm^{-1} (open triangles), and the background fluorescence of skin (open circles).
5 From the ratio of Raman to fluorescence intensity, shown as solid dots in the graph of Figure 7, an optimum excitation wavelength is seen to exist in the 500 nm wavelength region. The available strongest argon ion laser wavelengths of 4880 and 5145 Å are close to this optimum wavelength, and the required power levels can be easily achieved with a compact and relatively inexpensive air-cooled laser.

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Example 5

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In the course of performing Raman measurements, it was discovered that the fluorescence background of human skin bleached partially over a time period of several minutes. This effect is shown in the graph of Figure 8, where curve (a) corresponds to the fluorescence background immediately after exposure of a "fresh" skin spot, and curve (b) corresponds to the fluorescence background after a 7 minute exposure with 488 nm argon laser light, using a (safe) power density of 200 mW/cm^2 . While the shape of the fluorescence spectra remained unchanged, the intensity dropped to about 70% of its initial value. This value appears stable against further light exposure.

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The kinetics of the bleaching behavior of human skin fluorescence was further investigated. The results are shown in the graph of Figure 9, where the intensities of fluorescence at 525 nm versus time under irradiation with a 488 nm argon laser are plotted for the two excitation intensities, 200 mW/cm^2 (curve a) and 25 mW/cm^2 (curve b), respectively, showing that the fluorescence follows complicated non-exponential decay kinetics. Also shown in Figure 9 is the intensity versus time of the 1524 cm^{-1} carotenoid Raman peak (curve c), revealing that it remained substantially unchanged under the same power density level leading to fluorescence bleaching.

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Example 6

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The apparatus of Example 1 was used to measure the carotenoid content in various skin regions of the body of a healthy human volunteer. The graph of Figure 10 shows the fluorescence subtracted Raman spectral results from measurements of a finger (curve a) and the forehead (curve b) of the volunteer. The Raman response from the finger and, correspondingly, the carotenoid concentration, is seen to be about twice as high as the response from the forehead, showing that different levels of carotenoids are present in various skin areas of the body.

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Additional preliminary findings obtained from intact skin of volunteers indicate

that carotenoid levels vary significantly from person to person, even in the same body area. For example, it was found that the carotenoid levels of two white male foreheads differed by a factor of 38. Further preliminary data suggest that carotenoid levels decrease especially in smokers.

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Example 7

The apparatus of Example 1 was used to measure the carotenoid content in skin regions of the body of a human volunteer suffering from squamous cell carcinoma. The graph of Figure 11 shows the fluorescence subtracted Raman spectral results from measurements of a healthy skin region near the carcinoma (curve a), and the results from
10 measurements of the central region of the carcinoma (curve b). The peaks associated with the carotenoids are measured at 1015 cm^{-1} , 1159 cm^{-1} and 1524 cm^{-1} . The peak of curve (a) corresponds to a relatively high level of carotenoids in healthy skin, as indicated by the high photon count (intensity). The peak of curve (b) shows a much smaller photon count, indicating a lower level of carotenoids in carcinomic skin. Thus, a significant
15 difference appears to exist in the carotenoid concentration of both regions, with the carcinoma area having a diminished carotenoid content.

The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the
20 invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed is:

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1. A method for noninvasively determining the presence of a malignancy disease in biological tissue, comprising the steps of:

obtaining a light source which generates light at a wavelength that produces a Raman response with a wavelength shift for carotenoids to be detected;

directing light from the light source onto biological tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the tissue and does not substantially alter carotenoid levels in the tissue;

collecting light scattered from the tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the tissue;

filtering out the elastically scattered light; and

quantifying the intensity of the Raman signal, wherein a substantial difference between the intensity of the Raman signal and the intensity of Raman scattering from adjacent normal biological tissue indicates the presence or risk of a malignancy disease.

2. The method of claim 1, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

3. The method of claim 1, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

4. The method of claim 1, wherein the biological tissue resides in a live subject.

5. The method of claim 1, wherein the biological tissue is living skin.

6. The method of claim 5, wherein the light has an intensity of up to about 200 mW/cm².

7. The method of claim 5, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

8. The method of claim 1, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

9. A method for noninvasively determining the antioxidant status in biological tissue, comprising the steps of:

obtaining a light source which generates light at a wavelength that produces a Raman response with a wavelength shift for carotenoids to be detected;

directing light from the light source onto biological tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the tissue and does not substantially alter carotenoid levels in the tissue;

5 collecting light scattered from the tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the tissue;

 filtering out the elastically scattered light; and

 quantifying the intensity of the Raman signal in order to assess the
10 antioxidant status of the tissue.

10. The method of claim 9, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

11. The method of claim 9, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

15 12. The method of claim 9, wherein the biological tissue resides in a live subject.

13. The method of claim 9, wherein the biological tissue is living skin.

14. The method of claim 13, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

20 15. The method of claim 9, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

16. A method for noninvasive measurement of carotenoids in skin tissue, comprising the steps of:

 obtaining a light source which generates light at a wavelength that
25 produces a Raman response with a wavelength shift for carotenoids to be detected;

 directing light from the light source onto skin tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the skin tissue and does not substantially alter carotenoid levels in
30 the skin tissue;

 collecting light scattered from the skin tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the skin tissue;

 filtering out the elastically scattered light;

35 quantifying the intensity of the Raman signal; and

subtracting a background fluorescence signal of the skin tissue from the Raman signal of the carotenoids being detected.

17. The method of claim 16, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

5 18. The method of claim 16, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

19. The method of claim 16, wherein the light has an intensity of up to about 200 mW/cm².

10 20. The method of claim 16, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

21. The method of claim 16, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

22. An apparatus for noninvasive measurement of carotenoids and related chemical substances in biological tissue, comprising:

15 a laser light source that generates light at a wavelength giving a Raman response with a wavelength shift for carotenoids being detected;

a light delivery and collection module for directing light onto biological tissue and collecting scattered light from the tissue such that the light does not damage the tissue or substantially alter the carotenoid levels in the tissue;

20 a spectrally selective system for selecting Raman shifted light from the collected scattered light;

detection means for scanning and measuring the Raman shifted light at frequencies characteristic of carotenoids; and

25 quantifying means for determining Raman signal intensity for the carotenoids being detected.

23. The apparatus of claim 22, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

24. The apparatus of claim 22, wherein the spectrally selective system is a grating spectrometer.

30 25. The apparatus of claim 22, wherein the spectrally selective system includes a grating monochromator.

26. The apparatus of claim 22, wherein the spectrally selective system includes a holographic filter.

35 27. The apparatus of claim 22, wherein the spectrally selective system includes a dielectric filter.

28. The apparatus of claim 22, wherein the spectrally selective system includes an acousto-optic filter.

29. The apparatus of claim 22, wherein the detection means comprises a CCD detector array.

5 30. The apparatus of claim 22, wherein the detection means comprises an intensified CCD detector array.

31. The apparatus of claim 22, wherein the quantifying means comprises a personal computer.

10 32. The apparatus of claim 22, wherein the quantifying means comprises a CCD image display or monitor.

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AMENDED CLAIMS

[received by the International Bureau on 18 September 2000 (18.09.00);
original claims 1, 9, 12, 13 and 22 amended;
remaining claims unchanged (4 pages)]

1. A method for noninvasively determining the presence or risk of a malignancy disease in biological tissue, comprising the steps of:

obtaining a light source which generates light at a wavelength that produces a Raman response with a wavelength shift for carotenoids to be detected;

directing light from the light source onto biological tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the tissue and does not substantially alter carotenoid levels in the tissue;

collecting light scattered from the tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the tissue;

filtering out the elastically scattered light; and

quantifying the intensity of the Raman signal, wherein a substantial difference between the intensity of the Raman signal and the intensity of Raman scattering from adjacent normal biological tissue indicates the presence or risk of a malignancy disease.

2. The method of claim 1, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

3. The method of claim 1, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

4. The method of claim 1, wherein the biological tissue resides in a live subject.

5. The method of claim 1, wherein the biological tissue is living skin.

6. The method of claim 5, wherein the light has an intensity of up to about 200 mW/cm².

7. The method of claim 5, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

8. The method of claim 1, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

9. A method for noninvasively determining the antioxidant status in skin tissue, comprising the steps of:

obtaining a light source which generates light at a wavelength that produces a Raman response with a wavelength shift for carotenoids to be detected in skin tissue;

directing light from the light source onto skin tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the tissue and does not substantially alter carotenoid levels in the tissue;

collecting light scattered from the tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the tissue;

filtering out the elastically scattered light; and

quantifying the intensity of the Raman signal in order to assess the antioxidant status of the tissue.

10. The method of claim 9, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

11. The method of claim 9, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

12. The method of claim 9, wherein the skin tissue resides in a live subject.

13. The method of claim 9, wherein the skin tissue is living skin.

14. The method of claim 13, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

15. The method of claim 9, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

16. A method for noninvasive measurement of carotenoids in skin tissue, comprising the steps of:

obtaining a light source which generates light at a wavelength that produces a Raman response with a wavelength shift for carotenoids to be detected;

directing light from the light source onto skin tissue for which carotenoid levels are to be measured, the light having an intensity which does not cause destruction of the skin tissue and does not substantially alter carotenoid levels in the skin tissue;

collecting light scattered from the skin tissue, the scattered light including elastically and inelastically scattered light, the inelastically scattered light producing a Raman signal corresponding to carotenoids in the skin tissue;

filtering out the elastically scattered light;

quantifying the intensity of the Raman signal; and

subtracting a background fluorescence signal of the skin tissue from the

Raman signal of the carotenoids being detected.

17. The method of claim 16, wherein the light source generates light in a wavelength which overlaps the absorption bands of the carotenoids to be detected.

18. The method of claim 16, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

19. The method of claim 16, wherein the light has an intensity of up to about 200 mW/cm².

20. The method of claim 16, wherein the scattered light is measured at frequencies characteristic of carotenoids in the skin.

21. The method of claim 16, wherein the Raman signal is quantified via signal intensity calibrated with actual carotenoid levels.

22. An apparatus for noninvasive measurement of carotenoids and related chemical substances in biological tissue, comprising:

a laser light source that generates light at a wavelength giving a Raman response with a wavelength shift for carotenoids being detected;

a light delivery and collection module for directing light onto biological tissue and collecting scattered light from the tissue such that the light does not damage the tissue or substantially alter the carotenoid levels in the tissue, the light delivery and collection module comprising:

a first lens for collimating laser light;

a first narrow bandwidth filter in optical communication with the first lens;

first and second dichroic beam splitters in optical communication with the first narrow bandwidth filter;

a second lens adapted to direct a beam of laser light from the second dichroic beam splitter to tissue and collect scattered light from the tissue;

a second narrow bandwidth filter in optical communication with the second lens; and

a third lens in optical communication with the second narrow bandwidth filter;

a spectrally selective system for selecting Raman shifted light from the collected scattered light;

detection means for scanning and measuring the Raman shifted light at frequencies characteristic of carotenoids; and

quantifying means for determining Raman signal intensity for the carotenoids being detected.

23. The apparatus of claim 22, wherein the light source generates laser light in a wavelength range from about 450 nm to about 520 nm.

5 24. The apparatus of claim 22, wherein the spectrally selective system is a grating spectrometer.

25. The apparatus of claim 22, wherein the spectrally selective system includes a grating monochromator.

10 26. The apparatus of claim 22, wherein the spectrally selective system includes a holographic filter.

27. The apparatus of claim 22, wherein the spectrally selective system includes a dielectric filter.

28. The apparatus of claim 22, wherein the spectrally selective system includes an acousto-optic filter.

15 29. The apparatus of claim 22, wherein the detection means comprises a CCD detector array.

30. The apparatus of claim 22, wherein the detection means comprises an intensified CCD detector array.

20 31. The apparatus of claim 22, wherein the quantifying means comprises a personal computer.

32. The apparatus of claim 22, wherein the quantifying means comprises a CCD image display or monitor.

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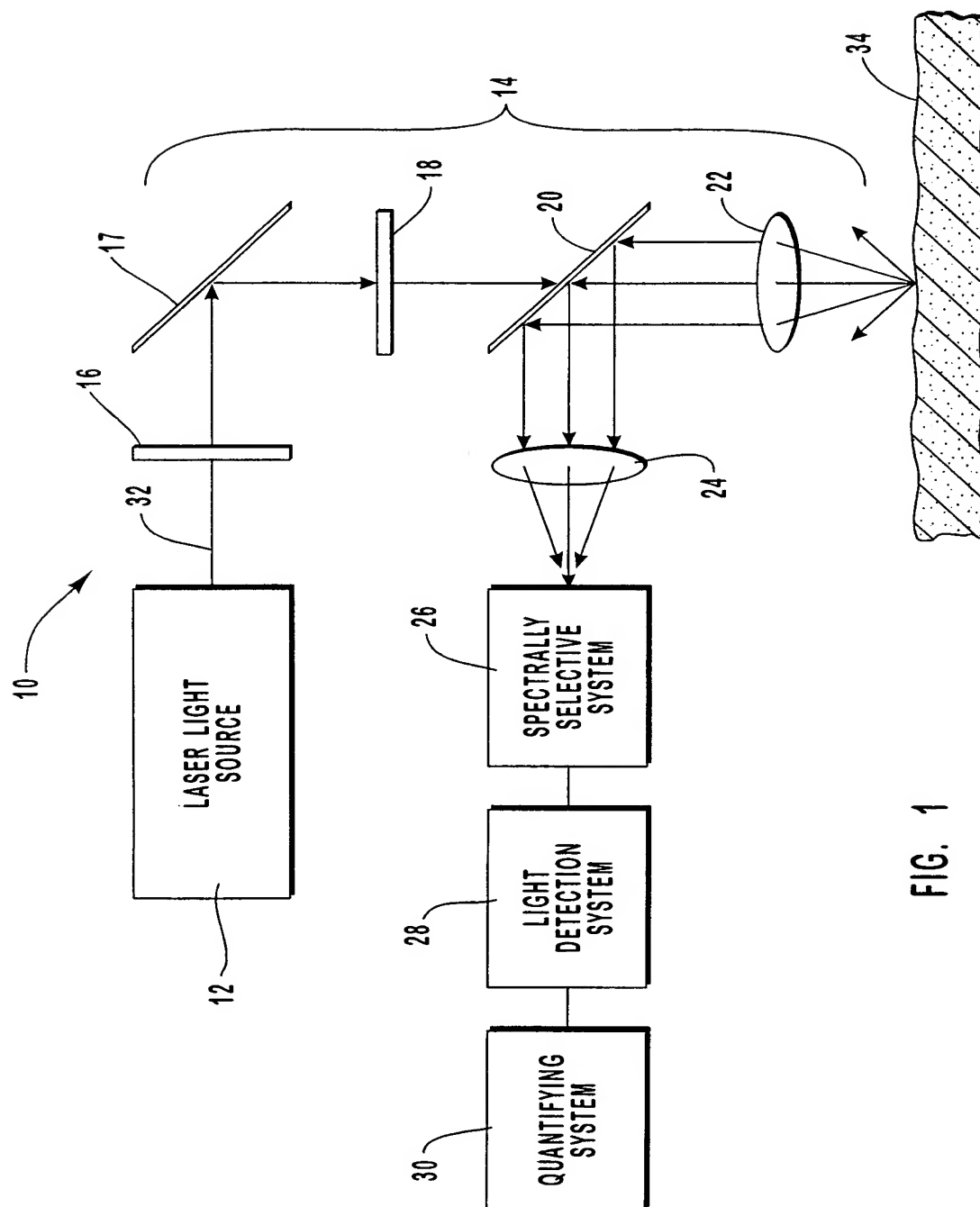


FIG. 1

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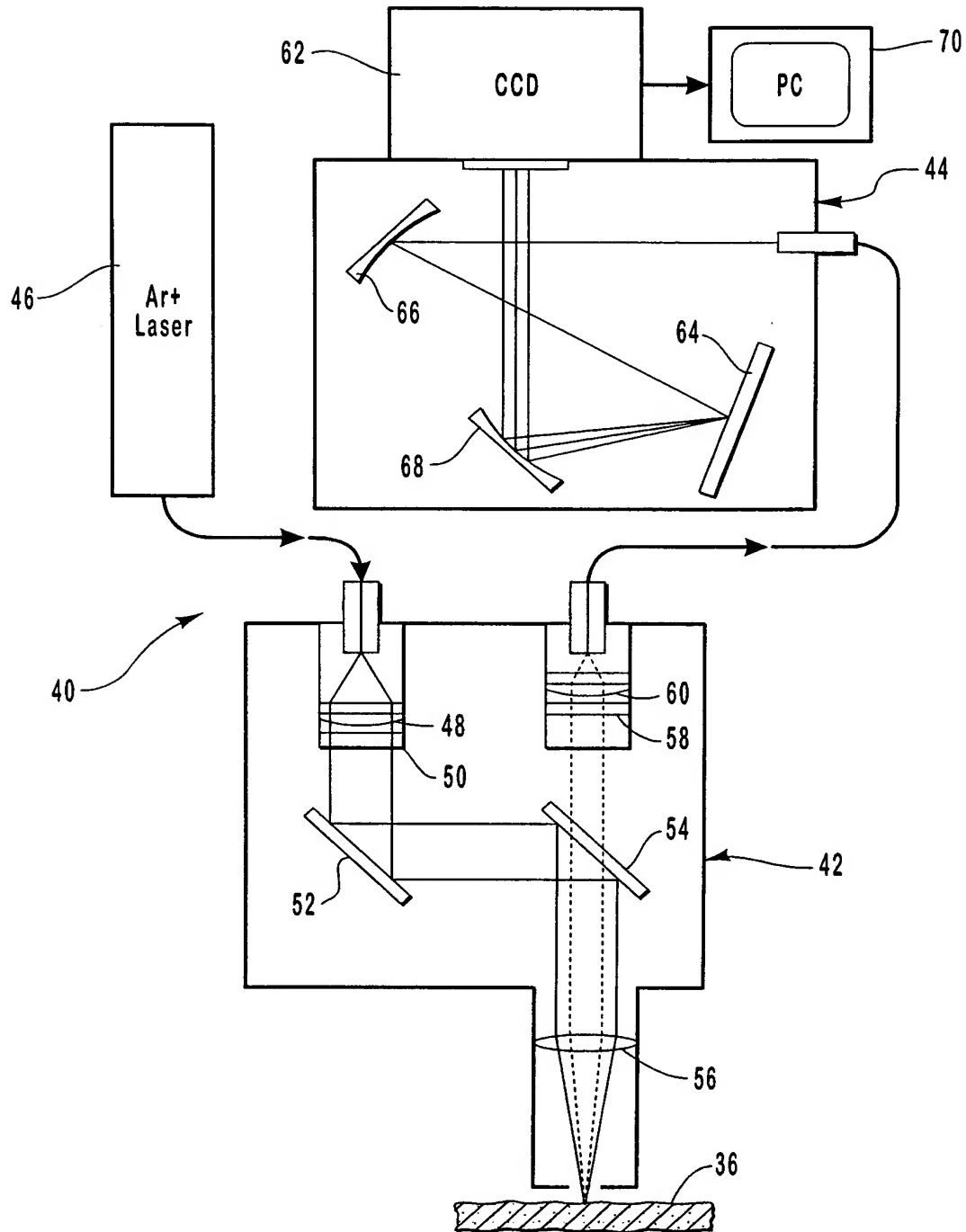


FIG. 2

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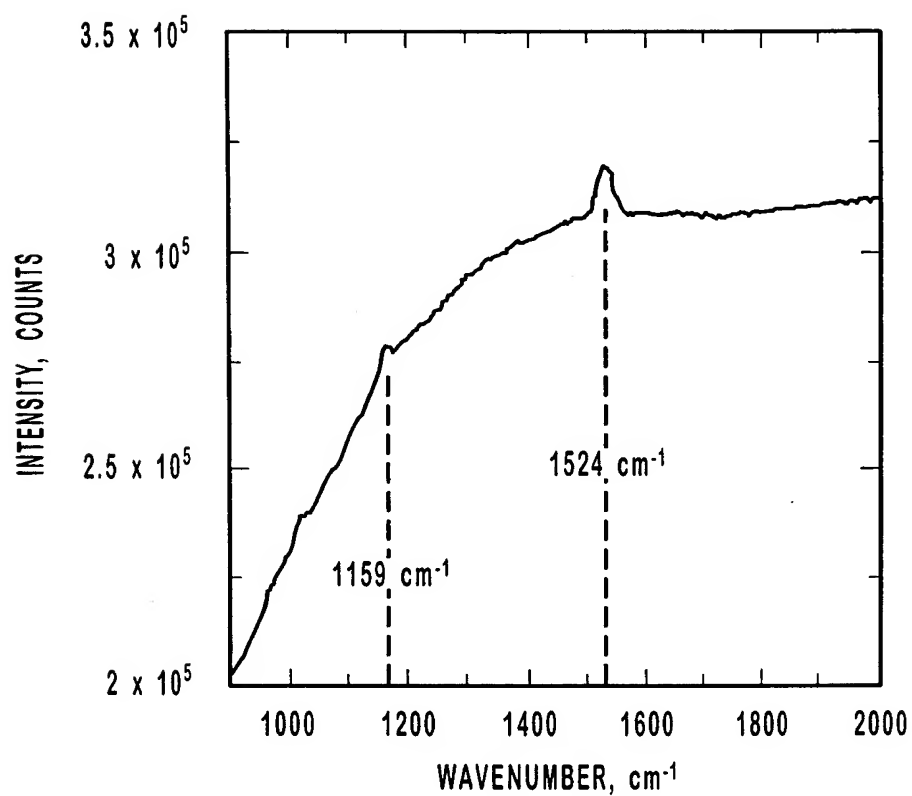


FIG. 3

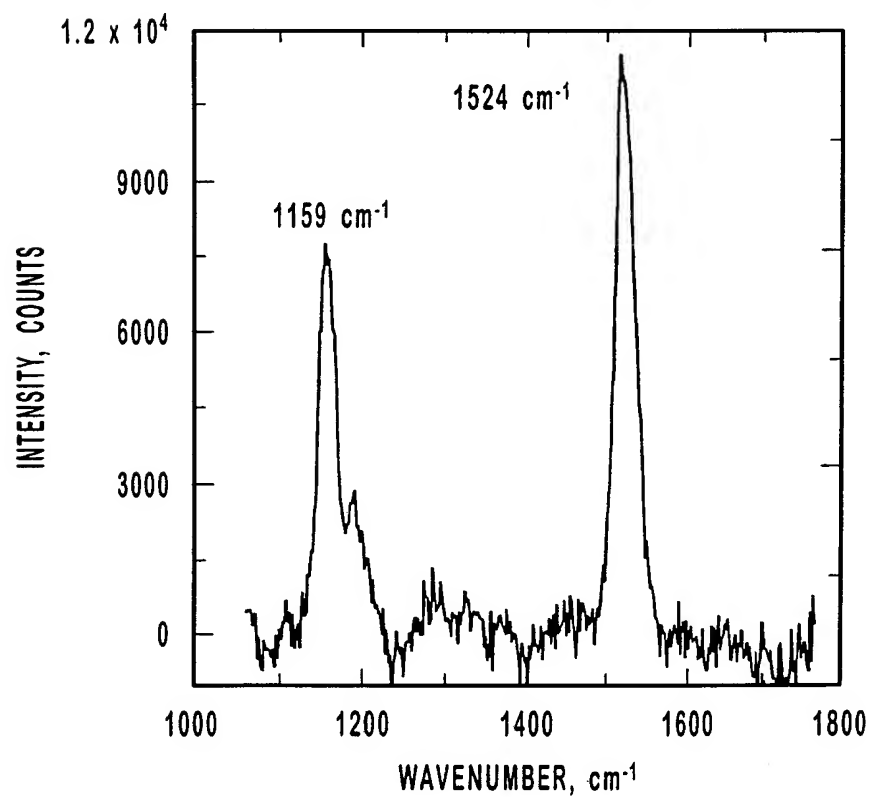


FIG. 4

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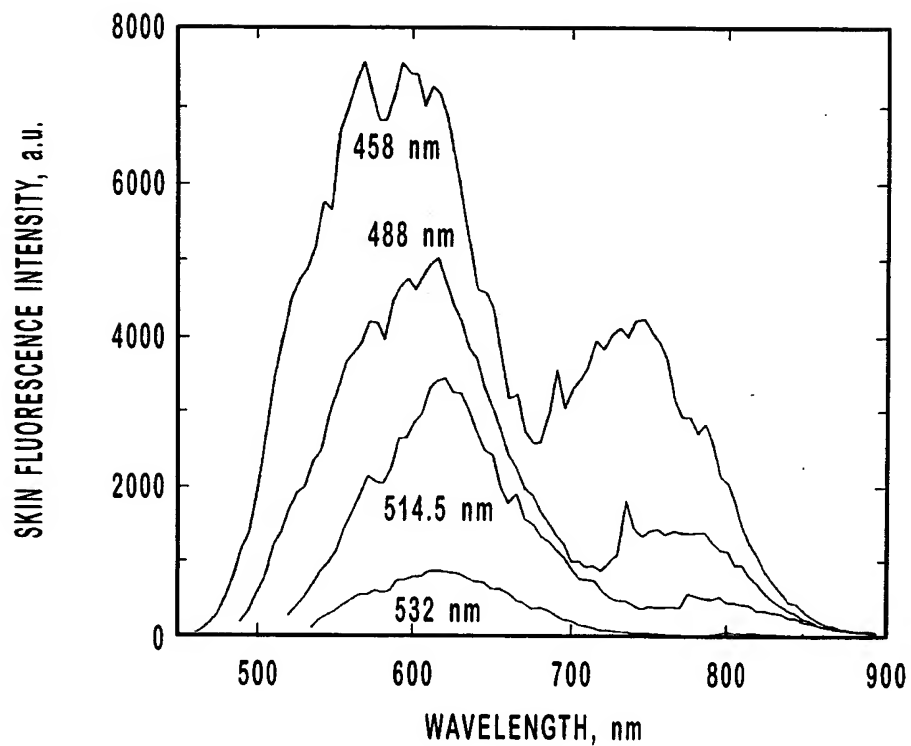


FIG. 5

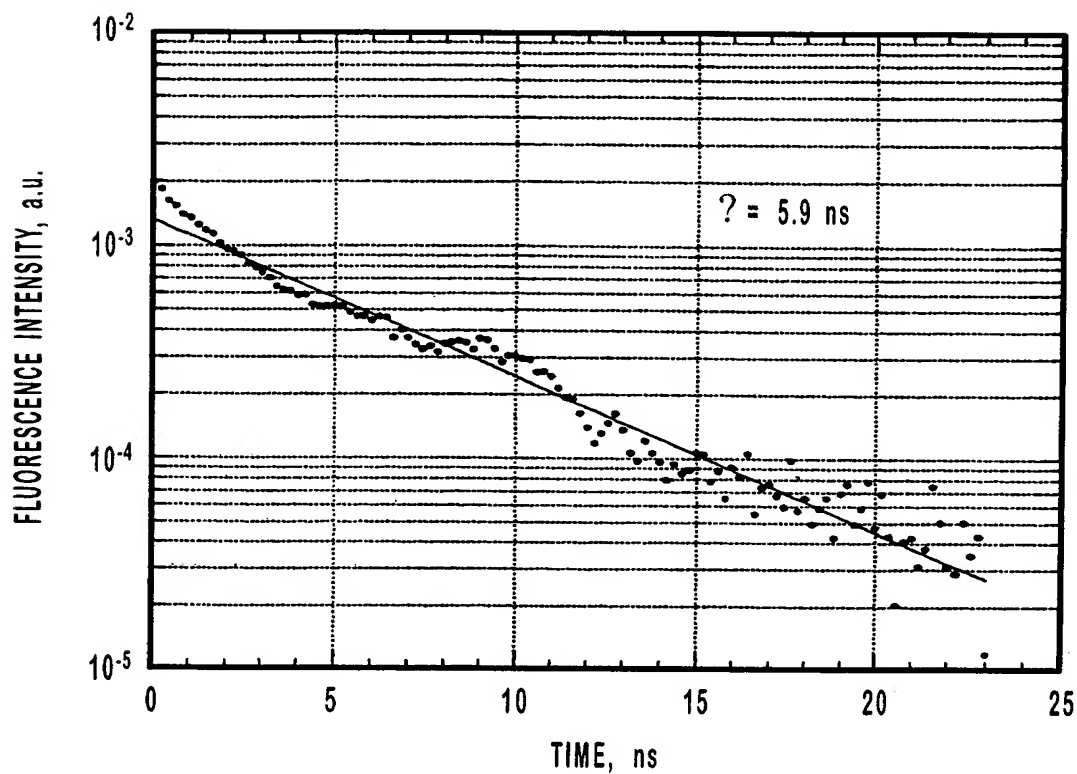


FIG. 6

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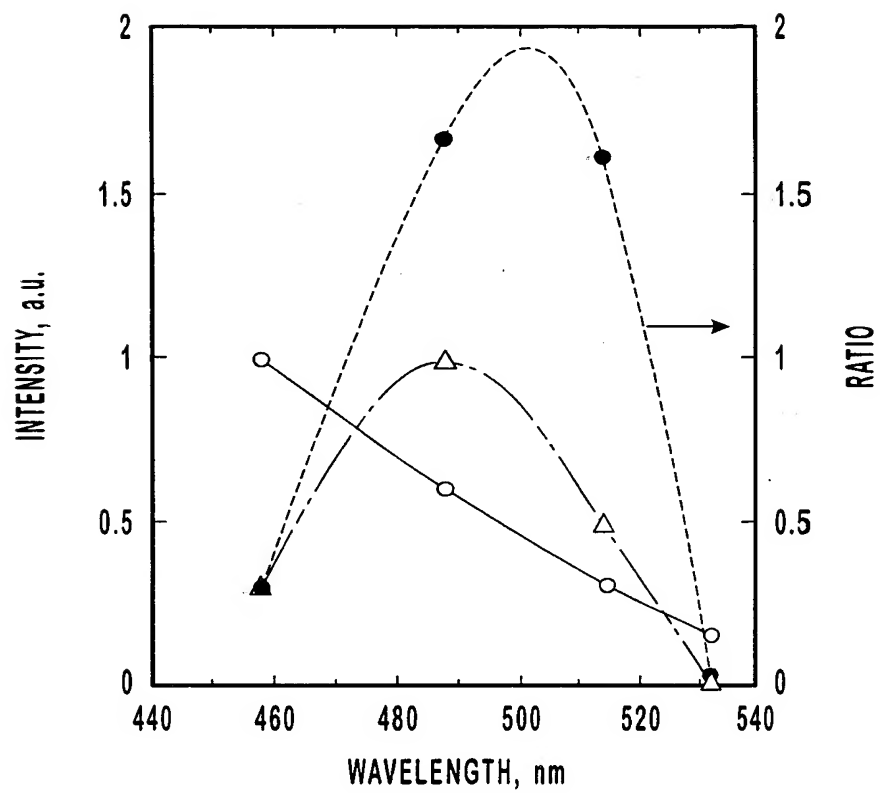


FIG. 7

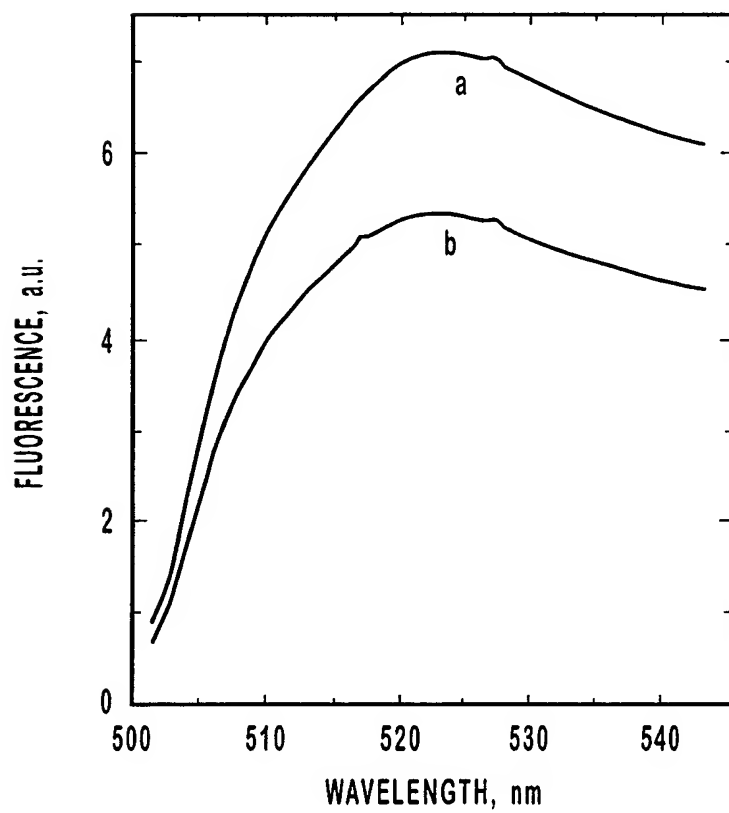


FIG. 8

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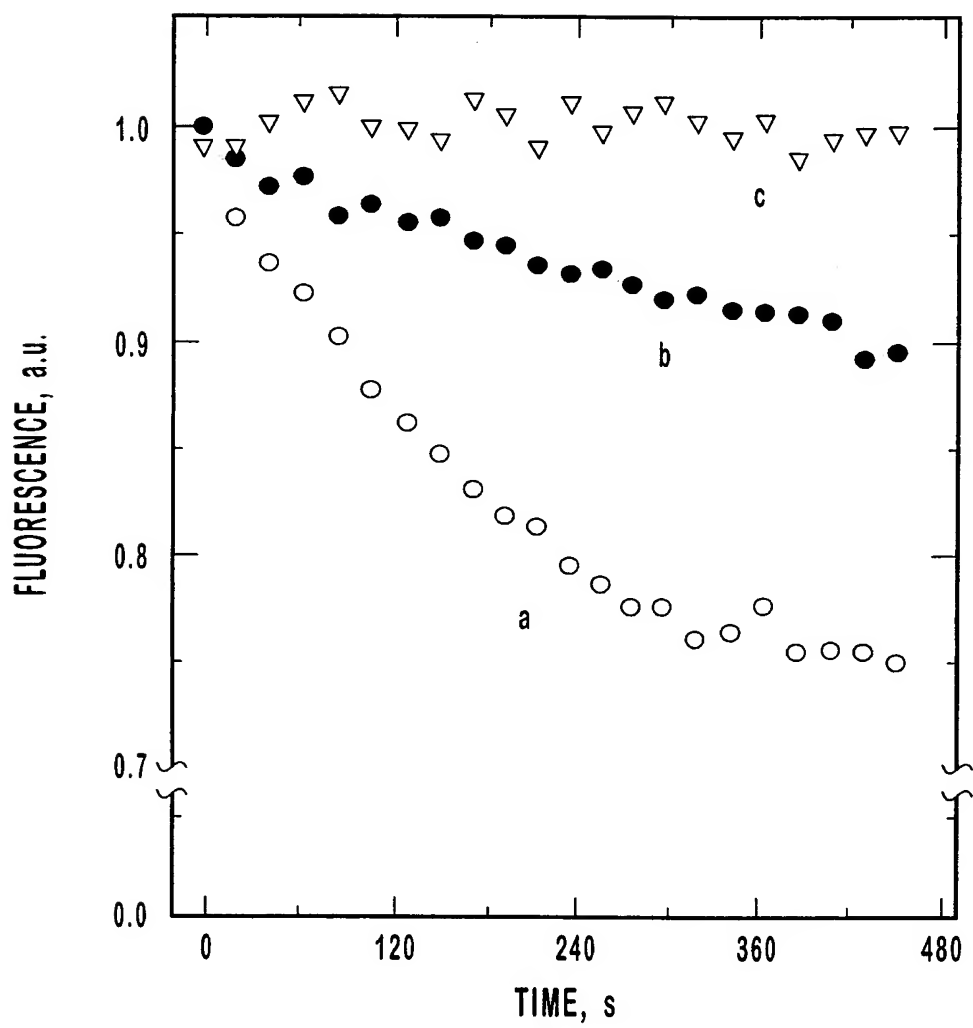


FIG. 9

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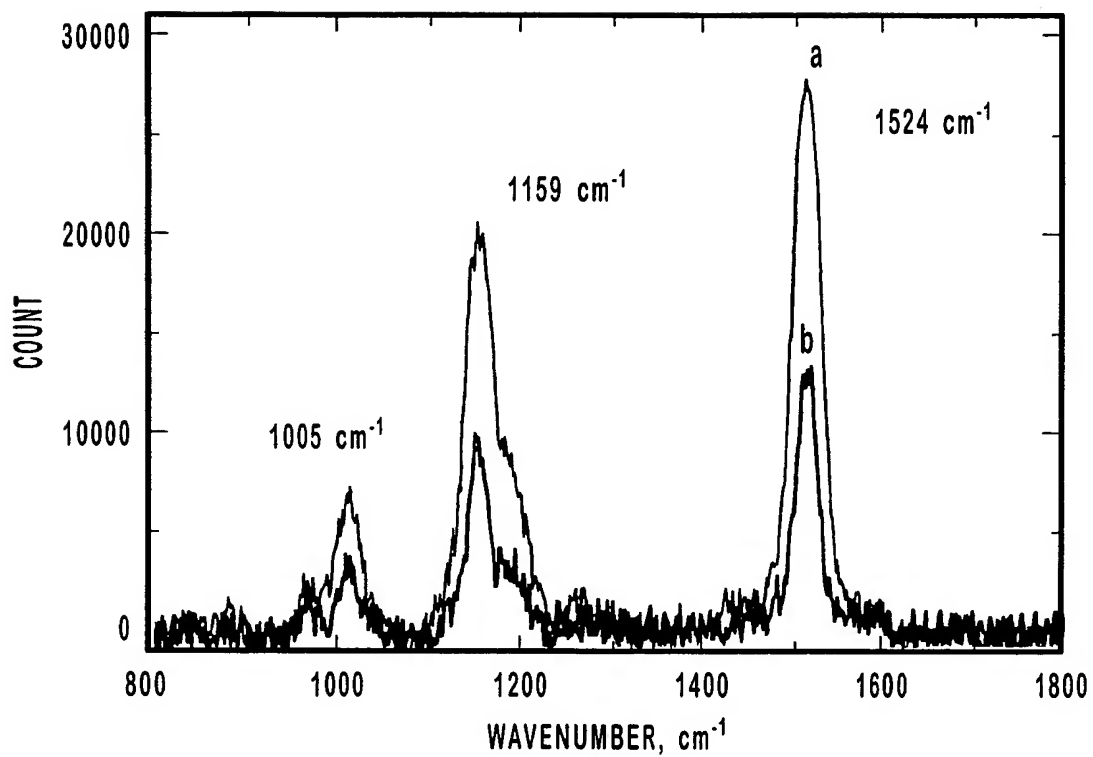


FIG. 10

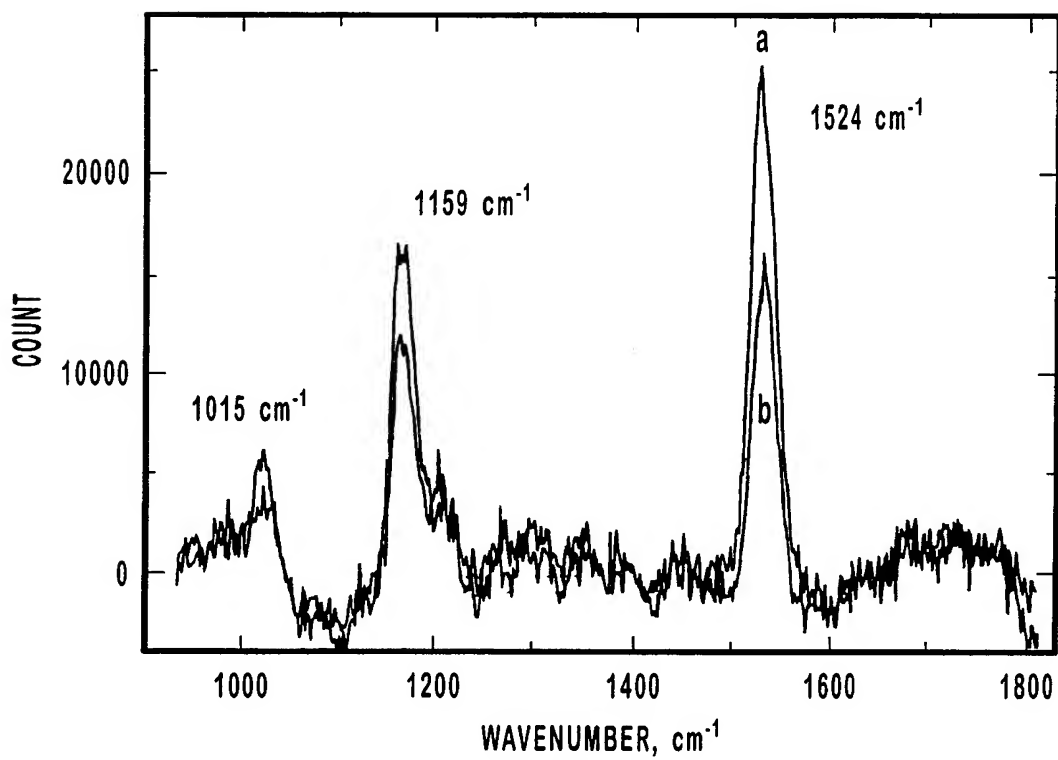


FIG. 11

INTERNATIONAL SEARCH REPORT

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U.S. : 356/301, 303; 600/310, 318, 473, 475-477; 606/2-4, 10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,873,831 A (BERNSTEIN et al.) 23 February 1999, entire document.	9-15, 22-32 ----- 1-8, 16-21
Y	US 5,697,373 A (RICHARDS-KORTUM et al.) 16 December 1997, entire document	1-8, 16-21

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

05 JUNE 2000

Date of mailing of the international search report

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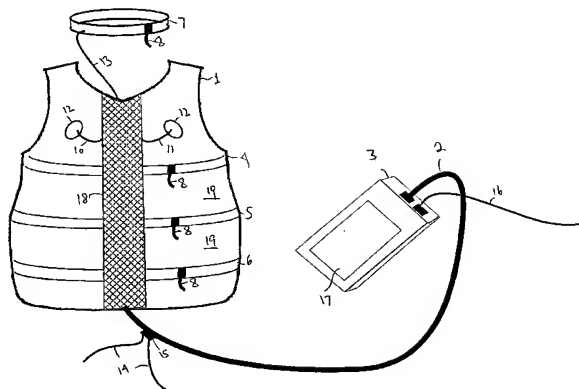
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYSTEMS AND METHODS FOR AMBULATORY MONITORING OF PHYSIOLOGICAL SIGNS



(57) Abstract: The present invention relates to the field of ambulatory and non-invasive monitoring of a plurality of physiological parameters of a monitored individual. The invention includes a physiological monitoring apparatus with an improved monitoring apparel worn by a monitored individual, the apparel having attached sensors for monitoring parameters reflecting pulmonary function, or parameters reflecting cardiac function, or parameters reflecting the function of other organ systems, and the apparel being designed and tailored to be comfortable during the individual's normal daily activities. The apparel is preferably also suitable for athletic activities. The sensors preferably include one or more ECG leads and one of more inductive plethysmographic sensors with conductive loops positioned closely to the individual to preferably monitor at least basic cardiac parameters, basic pulmonary parameters, or both. The monitoring apparatus also includes a unit for receiving data from the sensors, and for storing the data in a computer-readable medium. The invention also includes systems comprising a central data repository for receiving, storing, and processing data generated by a plurality of physiological monitored apparatuses, and for making stored data available to the individual and to the health care providers.



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SYSTEMS AND METHODS FOR AMBULATORY MONITORING OF PHYSIOLOGICAL SIGNS

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1. FIELD OF THE INVENTION

The present invention relates to the field of ambulatory and non-invasive monitoring of an individual's physiological parameters. In particular, the invention relates to a monitoring apparatus with an improved apparel worn by a monitored individual, the apparel having attached sensors for monitoring parameters reflecting pulmonary function, or
10 parameters reflecting cardiac function, or parameters reflecting the function of other organ systems. The invention also includes systems for receiving, storing, and processing physiological-parameter data, and for making it available to the individual and to health care providers.

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2. BACKGROUND OF THE INVENTION

In the following, the term "plethysmography" (and its derivative words) means measurement of a cross-sectional area of the body, such as a cross-sectional area of the chest or of the abdomen, or a body part, such as a cross-sectional area of the neck or of an arm. (This meaning is somewhat more limited than is standard in the medical arts.)
20 Further, the phrase "inductive plethysmography" means herein plethysmographic measurements which depend on inductance determinations.

Measurement of pulmonary and cardiac physiological parameters by means of inductive plethysmography is known. For example, many measurement methods and apparatus are disclosed in the following U.S. patents, the entire disclosures of which are
25 incorporated herein, in their entireties, by reference, for all purposes.

(1) The '872 Patent: U.S. patent no. 4,308,872, issued Jan. 5, 1982 and titled "Method and Apparatus for Monitoring Respiration," discloses a method and apparatus for monitoring respiration volumes by measuring variations in the patient's chest cross sectional area, or variations in both chest and abdomen cross sectional areas, each area being
30 measured by determining the inductance of an extensible electrical conductor closely looped around the body, and the measurements being calibrated by measuring the area variations for a few breaths while directly measuring corresponding volumes of breath, preferably while the patient assumes at least two body positions, for example sitting and supine.

(2) The '534 Patent: U.S. patent no. 4,373,534, issued Feb. 15, 1983 and titled
35 "Method and Apparatus for Calibrating Respiration Monitoring System," discloses methods and systems in which respiration volume is determined by weighting signals representing

abdominal and chest cross-sectional areas, where the weighting factors are determined by a procedure involving measuring respiration volume by an alternate measuring apparatus along with unweighted chest and abdomen signals, the measurements occurring for a first series of breaths based with a first relative chest and abdominal contribution and for a
5 second series of breaths based on a second relative chest and abdominal contribution.

(3) The '252 Patent: U.S. patent no. 4,452,252, issued Jun. 5, 1984 and titled "Non-invasive Method for Monitoring Cardiopulmonary Parameters," discloses a method for monitoring cardiopulmonary events by inductive plethysmographic measurement of a cross-sectional area of the neck, and further discloses a method for monitoring mouth
10 volume by inductive plethysmographic measurement of a cross-sectional area of the head in a plane which extends through the mouth.

(4) The '015 Patent: U.S. patent no. 4,456,015, issued Jun. 26, 1984 and titled "Non-invasive Method for Semiquantitative Measurement of Neck Volume Changes," discloses a method of plethysmographic measurement of a subject's neck volume by
15 providing an extensible conductor closely circling the neck and, first, calibrated against cross-sectional area so that neck volume may be determined from the conductor's inductance, and also, second, calibrated against invasively-measured intrapleural pressure so that the intrapleural pressure may also be determined from the conductor's inductance, and also so that intrapleural pressure may also be obtained from measured neck volume.

(5) The '407 Patent: U.S. patent no. 4,648,407, issued Mar. 10, 1987 and titled "Method for Detecting and Differentiating Central and Obstructive Apneas in Newborns," disclosing methods for detecting the presence and origin of apnea in newborns by concurrently monitoring relative movement of the cranial bones (which have been found to move with respiration as a function of intrapleural pressure), preferably by a surface
25 inductive plethysmographic transducer, and nasal ventilation, preferably by a nasal cannula, thermistor, thermocouple or CO₂ sensor, wherein absence of changes in both cranial bone movement and respiratory air flow at the nose indicates of the presence of central apnea, while absence of nasal air flow accompanied by continuing cranial bone movements indicates of obstructive apnea.

(6) The '962 Patent: U.S. patent no. 4,777,962, issued Oct. 18, 1988 and titled "Method and Apparatus for Distinguishing Central Obstructive and Mixed Apneas by External Monitoring Devices Which Measure Rib Cage and Abdominal Compartmental Excursions During Respiration," discloses an apparatus and method for distinguishing between different types of apneic episodes. The method includes measuring a new index,
35 Total Compartmental Displacement/Tidal Volume (TCD/VT), and measuring the phase relation between the abdominal and rib cage contributions to total respiration volume,

wherein an episode is classified as central, obstructive or mixed based on the value of TCD/VT and the phase relation.

(7) The '640 Patent: U.S. patent no. 4,807,640, issued Feb. 28, 1989 and titled "Stretchable Band-type Transducer Particularly Suited for Respiration Monitoring Apparatus," discloses an improved, low-cost stretchable band incorporating a conductor for disposition about the human torso or other three dimensional object, and particularly intended for use with respiration monitoring by means of inductive plethysmography, a method for making the band, which method is suitable to mass production techniques, and an improved enclosure housing circuitry releasably connected to the conductor in the band when the band is incorporated in respiration monitoring apparatus.

(8) The '473 Patent: U.S. patent no. 4,815,473, issued Mar. 28, 1989 and titled "Method and Apparatus for Monitoring Respiration," discloses a method and apparatus for monitoring respiration volumes by inductive plethysmographic measurement of variations in a patient's chest cross sectional area, or preferably, variations in both chest and abdomen areas during breathing, and a method for calibrating such an apparatus by measuring cross-sectional area variations for a few breaths while directly measuring corresponding volumes of breath, preferably while the patient assumes at least two body positions, for example sitting and supine.

*** (9) The '766 Patent: U.S. patent no. 4,860,766, issued Aug. 29, 1989 and titled "Noninvasive Method for Measuring and Monitoring Intrapleural Pressure in Newborns," discloses measuring intrapleural pressure of a newborn subject by detecting relative movement between adjacently-proximate cranial bones, preferably, using a surface inductive plethysmographic transducer secured on the subject's head across at least two adjacently-proximate cranial bones, and a method of calibrating such measurements by temporarily manually occluding the subject's nose or, if intubated, the endotracheal tube, to measure the airway pressure during such occlusion as the subject makes an inspiratory effort and comparing the measured pressure to the measured signal.

(10) The '109 Patent: U.S. patent no. 4,834,109, issued May 30, 1989 and titled "Single Position Non-invasive Calibration Technique," discloses an improved method for calibrating inductive plethysmographic measurement of respiration volume by totaling, during a period of breathing, a plurality of values of a parameter indicative of the relative amplitude, for each breath, of uncalibrated rib cage and abdomen signals, and by dividing the average variability of the means of the total of the values of one of the rib cage and abdomen signals by the average variability of the mean of the total of the values of the other signal, the quotient being so derived represents a signal weighting factor for determining respiration volume.

(11) The '277 Patent: U.S. patent no. 4,986,277, issued Jan. 22, 1991 and titled "Method and Apparatus for Non-invasive Monitoring of Central Venous Pressure," discloses a method and apparatus for measuring central venous pressure (CVP) and changes in CVP along with an improved transducer (50) for measuring CVP in infants, wherein a plethysmographic transducer is disposed on the neck of a subject (or on the head in the case of infants), the signal from the transducer is processed to obtain a cardiac component, and the vertical distance from the transducer to a reference level is adjusted until a position is located at which the signal changes between a venous configuration and an arterial or mixed venous-arterial configuration, at which position the vertical distance approximates CVP.

(12) The '540 Patent: U.S. patent no. 5,040,540, issued Aug. 20, 1991 and titled "Method and Apparatus for Non-invasive Monitoring of Central Venous Pressure, and Improved Transducer Therefor," discloses an improved method and apparatus for measuring central venous pressure (CVP), and changes in CVP, along with an improved transducer for measuring CVP in infants.

(13) The '935 Patent: U.S. patent no. 5,159,935, issued Nov. 3, 1992 and titled "Non-invasive Estimation of Individual Lung Function," discloses a non-invasive method and apparatus for plethysmographic monitoring individual lung function by disposing a transducer on the torso above the lung to be monitored, the transducer producing a signal corresponding to movement of the torso portion there beneath which, in turn, corresponds to changes in the volume of the underlying lung, and also a method and apparatus for monitoring regional lung volume changes by utilizing transducers positioned on the torso to encompass only a portion of the underlying lung.

(14) The '151 Patent: U.S. patent no. 5,178,151, issued Jan. 12, 1993 and titled "System for Non-invasive Detection of Changes of Cardiac Volumes and Aortic Pulses," discloses a method and an apparatus therefor for monitoring cardiac function in an animal or human subject including the steps of placing a first movement detecting transducer on the torso, said transducer overlying at least part of two diametrically opposed borders of the heart or great vessels; generating a signal indicative of the movement of the torso portion subtended by the transducer, said signal including a cardiac component comprising at least a segmental ventricular volume waveform or a segmental aortic pressure pulse waveform and assessing cardiac function by monitoring changes in said ventricular volume waveform or said aortic pressure pulse waveform.

(15) The '678 Patent: U.S. patent no. 5,301,678, issued Apr. 12, 1994 and titled "Stretchable Band-Type Transducer Particularly Suited for Use with Respiration Monitoring Apparatus," an improved, low-cost stretchable band incorporating a conductor for

disposition around the human torso or other three-dimensional object, and particularly intended for use with plethysmographic respiration monitoring apparatus, is disclosed.

(16) The '968 Patent: U.S. patent no. 5,331,968, issued Jul. 26, 1994 and titled "Inductive Plethysmographic Transducers and Electronic Circuitry Therefor," discloses an apparatus and method for improving the detection of the inductance "signal" generated by an inductive plethysmograph by modifying the design of the inductive plethysmograph and also by improving the design of the associated circuitry, both of which permit the associated circuitry may be located remotely rather than on the transducer, the improvement including selecting the impedance matching transformer joining an inductive plethysmograph to an oscillator such that the inductance of its primary winding is greater than about ten times the reflected inductance of the inductive plethysmograph and the cable joining it to the transformer, or circling the conductor of the inductive plethysmograph therein around the relevant body portion a plurality of times, or selecting the cable connecting the inductive plethysmograph to the transformer such that the ratio of the diameter of its screen to the diameter of its center conductor is minimized for reducing the inductance per unit length thereof.

(17) The '425 Patent: U.S. patent no. 5,588,425, issued Dec. 31, 1996 and titled "Method and Apparatus for Discriminating Between Valid and Artifactual Pulse Waveforms in Pulse Oximetry," discloses a method and apparatus for use in pulse oximetry for discriminating between valid pulse waveforms, determined with a photoelectric plethysmograph, from which arterial oxygen saturation levels are accepted, and artifactual pulse waveforms, from which saturation levels are rejected, according to whether the systolic upstroke time of each pulse waveform is within a predetermined range, it having been discovered that systolic upstroke times for valid pulse waveforms are in a consistent, narrow range which varies only slightly from subject to subject and which may be defined empirically for each subject or established by a default setting applicable to all subjects,

(18) The '388 Patent: U.S. patent no. 6,015,388, issued Jan. 18, 2000 and titled "Method for Analyzing Breath Waveforms as to Their Neuromuscular Respiratory Implications," discloses a method for measuring respiratory drive by determining a peak inspiratory flow and a peak inspiratory acceleration from a breath waveform derived from rib cage motion and abdominal motion measured by external respiratory measuring devices, such as those based on inductive plethysmography, the measured respiratory drive being usable to initiate inspiration by a mechanical ventilator and for determining an index describing a shape of the waveform for controlling a continuous positive air pressure (CPAP) device.

(19) The '203 Patent: U.S. patent no. 6,047,203, issued Apr. 4, 2000 and titled "Physiologic Signs Feedback System," discloses a non-invasive physiologic signs monitoring device which includes a garment, in a preferred embodiment, a shirt, with electrocardiogram electrodes and various inductive plethysmographic sensors sewn, embroidered, embedded, or otherwise attached to the garment with an adhesive, signals generated by the sensors being transmitted to a recording/alarm device where they are logged and monitored for adverse or other preprogrammed conditions, which is signaled by When an adverse condition or other preprogrammed condition occurs, a message is communicated to the patient by either an audio message or a display. The recording/alarm unit is also connectable to a remote receiving unit for monitoring by a health care professional or other machine.

However, nowhere in the art of inductive plethysmography are found teachings of practical and effective apparatus for non-invasive, ambulatory monitoring, of pulmonary and cardiac parameters. Such practical and effective monitoring apparatus would be of great benefit by assisting the transfer of health care from traditional hospital-based care, which is administered by trained health care workers, to home-based self care, which is administered by the individual patient during, if possible, the patient's normal daily activities. This transfer in health care has been found socially desirable because it may reduce health care costs and may increase patient involvement in and commitment to their treatment plans. Non-invasive and ambulatory monitoring apparatus may assist this transfer, because it eliminates the risks associated with invasive sensors placed within the body, such as intravascular catheters, risks which are considerably heightened outside of the hospital.

Citation or identification of any reference in this Section, including the patents listed above, or in any section of this application shall not be construed that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention has for its objects practical and effective apparatus for non-invasive and ambulatory monitoring of key pulmonary and cardiac parameters along with a system that may be used for interpretation and use of monitoring data to improve health care outcomes and to reduce health case costs. In preferred embodiments, the preferred apparatus is a garment which, while including inductive plethysmographic and other physiologic sensors, is sufficiently comfortable and unobtrusive to be worn for most activities of daily life.

In more detail, in a first embodiment, the present invention includes a monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising: a monitoring garment comprising a shirt for the torso of the individual to be monitored, one or more inductive plethysmographic (IP) sensors, each IP sensor comprising
5 an inductance sensor including at least one conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop, a cardiac cycle sensor for generating signals responsive to occurrence of cardiac ventricular contractions, a signal cable for carrying signals from the sensors, and a microprocessor unit comprising a microprocessor for
10 receiving signals from the signal cable and for recording digital data derived from all received signals in a removable computer-readable memory media.

In first aspects of the first embodiment, the cardiac cycle sensor comprises at least one electrocardiogram (ECG) electrode attached to the individual to be monitored; the cardiac cycle sensor comprises at least one IP sensor closely fitting about the neck of the
15 individual to be monitored, wherein signals the inductance of the IP sensor is responsive to cardiac ventricular contractions because the cross-sectional area of the neck is responsive to carotid artery pulsations generated by cardiac ventricular contractions and the inductance of the IP sensor is responsive to the cross-sectional area of the neck; the computer-readable medium comprises a magnetic disk; the computer-readable medium comprises a flash
20 memory module (64 MB or more).

In second aspects of the first embodiment, the monitoring garment further comprises a band for the neck of the individual to be monitored, and the IP sensors comprise a neck inductive plethysmographic sensor operatively arranged for generating signals responsive to jugular venous pulse, carotid arterial pulse, respiration-related intra-pleural pressure
25 changes, contraction of neck muscles, and swallowing deflections, and the signal cable further comprises an attachment to the conductive loop of the neck IP sensor; the IP sensors comprise at least one abdominal IP sensor including one or more conductive loops and at least one rib cage IP sensor including one or more conductive loops operatively arranged for measuring breathing patterns of the patient; the IP sensors comprise at least one thoracic IP
30 sensor including a two or more conductive loops operatively arranged for measuring ventricular stroke volume; the IP sensors comprise at least one lower abdominal IP sensor operatively arranged for measuring intra-lower-abdominal contractions and dilations; the IP sensors comprise at least one two hemithoracic IP sensors operatively arranged for measuring breathing and paradoxical motion between two hemithoraces of the patient.

35 In third aspects, the first embodiment further comprises one or more further sensors attached to the signal cable and selected from a group comprising a body position sensor for

indicating a posture of the individual, a pulse oximeter for indicating arterial oxygenation saturation, and a throat microphone for indicating talking and snoring; or at least two body position sensors, a first body position sensor mounted on the garment and a second body position sensor mounted on a thigh of the individual; and the IP inductive plethysmographic sensors are attached to the garment as an integral part of the garment via an attachment consisting of one of sewing, embroidering, embedding, weaving and printing the inductive plethysmographic sensor into the garment; the microprocessor unit further comprises an audio device for generating audio indications to the individual being monitored; the microprocessor unit further comprises a display unit for displaying viewable messages to the individual being monitored; the microprocessor unit further comprises an input unit for the individual being monitored to input information or commands to the microprocessor unit.

In fourth aspects of the first embodiment, the microprocessor unit further comprises a memory accessible to the microprocessor, and wherein the memory comprises encoded software instructions for causing the microprocessor to read input data and to write output data derived from the input data in the removable computer-readable memory media; the memory further comprises encoded software instructions for causing the microprocessor to determine significant physiological events in the individual being monitored and to indicate audibly determined significant events to the individual; the microprocessor unit comprises components for wirelessly transmitting determined events and the memory further comprises encoded software instructions for causing the microprocessor to determine significant temporal physiological trends in the individual being monitored and to indicate audibly determined significant trends to the individual; the microprocessor unit comprises components for wirelessly transmitting determined significant trends; the memory further comprises encoded software instructions for causing the microprocessor to compress data before writing to the removable computer-readable memory media.

In fifth aspects of the first embodiment, the microprocessor unit further comprises circuitry for deriving digital data from non-digital data received from the signal cable; the monitoring apparatus further comprises circuitry for generating a variable-frequency signal from each IP sensor, the generated frequency being responsive to the inductance of the conductive loop of the IP sensor, and wherein the microprocessor unit further comprises circuitry for deriving digital data from the generated variable-frequency signals, the digital data comprising encoding of the variable frequency of the signals with errors of 100 ppm or less.

In a second embodiment, the present invention includes a monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising: a

monitoring garment comprising a shirt for the torso of the individual to be monitored, one or more inductive plethysmographic (IP) sensors, each IP sensor comprising (i) a longitudinal band of elastic material attached to the garment for closely encircling the torso, (ii) an inductance sensor including at least one flexible conductive loop attached to the longitudinal band, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop, and (iii) a tightening device for adjusting circumferential tightness of the IP sensor to substantially prevent longitudinal movement of the IP sensor along the torso, and a microprocessor unit comprising a microprocessor for receiving signals from the IP sensors and for recording digital data derived from all received signals in a removable computer-readable memory media.

In first aspects of the second first embodiment, longitudinal motion of each IP sensor is substantially prevented when the physiological parameters indicated by the inductance of the conductive loop of the sensor do not measurably change; the monitoring garment comprises excess fabric arranged to permit longitudinal stretching of the torso without applying force to the IP sensors sufficient to cause substantial longitudinal motion; longitudinal motion of each IP sensor is substantial if physiological parameters indicated by the inductance of the conductive loop of the sensor change as the monitoring garment is worn by the individual; the monitoring garment comprises fabric with sufficient longitudinal elasticity to permit longitudinal stretching of the torso without applying force to the IP sensors sufficient to cause substantial longitudinal motion.

In second aspects of the second embodiment, the tightening device comprises a cinch band and a gripping device for releasably gripping excess cinch band under tension; the tightening device comprises a drawstring;

In third aspects, the second embodiment, comprises a cardiac timing sensor for generating signals responsive to cardiac ventricular contractions, and wherein the microprocessor unit further records digital data derived from signals received from the cardiac timing sensor; or a signal cable for carrying signals from the sensors to the microprocessor unit.

In a third embodiment, the present invention includes a monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising: a monitoring garment comprising a shirt for the torso of the individual to be monitored and a longitudinal fastener for opening and closing the shirt, one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop, a cardiac timing sensor for generating signals responsive to

occurrence of cardiac ventricular contractions, a signal cable for carrying signals from the sensors comprising at least one module, wherein the module is coupled to and electrically completes the conductive loops of the IP sensors, wherein termini of the conductive loops may be uncoupled from module, and wherein the module comprises circuitry for generating
5 signals responsive to the IP sensors, and a microprocessor unit comprising a microprocessor for receiving signals from the signal cable and for recording digital data derived from all received signals in a removable computer-readable memory media.

In first aspects of the third embodiment, at least one IP sensor further comprises a tightening device for adjusting circumferential tightness of the IP sensor to substantially
10 prevent longitudinal movement of the IP sensor along the torso, and wherein the tightening device can be arranged not to impede unfastening of the shirt; the conductive loops of the IP sensors and the module further comprise mating connectors so that the conductive loops may be connected and disconnected from the module; the signals generated by the module in response to each IP sensor comprise digital data encoding the frequency of an oscillator
15 responsive to the inductance of the conductive loop of the IP sensor, the frequency being encoded with errors of 100 (or 10) ppm or less;

In second aspects of the third embodiment, the signals generated by the module in response to each IP sensor comprise signals of variable frequency, the frequency being responsive to the inductance of the conductive loop of the IP sensor; the microprocessor
20 unit further comprises circuitry for deriving digital data from the variable-frequency signals generated from each IP sensor, the digital data comprising encoding of the variable frequency of the signals with errors of 100 ppm or less; the microprocessor unit further comprises multiplex circuitry for permitting single deriving circuitry to derive digital data from a plurality of variable-frequency signals.

25 In a fourth embodiment, the present invention includes a monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising: a monitoring garment comprising a shirt for the torso of the individual to be monitored, one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso,
30 wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop, a cardiac timing sensor for generating signals responsive to occurrence of cardiac ventricular contractions, a signal cable for carrying signals directly from the conductive loops of the IP sensors and for carrying signals from the sensor, electronic circuitry comprising (i) a multiplexing switch for connecting the conductive loop
35 of any one of the IP sensors to an oscillator, the oscillator having an oscillation frequency responsive to the inductance of the conductive loop connected by the multiplexing switch,

and (ii) a demodulator operatively coupled to the oscillator and outputting digital data responsive to the oscillation frequency, and a microprocessor unit comprising a microprocessor for receiving signals from the signal cable and for receiving digital data from the electronic circuitry and for recording digital data from received inputs in a
5 removable computer-readable memory media.

In first aspects of the fourth embodiment, the digital data responsive to the oscillation frequency has errors of 100 (or 10) ppm or less; the electronic circuitry is housed in the microprocessor unit; the resistance of the data signal cables and the multiplexing switch from the conductive loop of any IP sensor to the oscillator is less than 1 Ω ; the
10 multiplexing switch is controlled so that oscillator is periodically connected to the conductive loop of each IP sensor for the duration of a sampling period (1 msec or less).

In second aspects of the fourth embodiment, the digital data output by the demodulator comprises digital data encoding a count of a number cycles of the oscillator occurring within a sampling period and digital data encoding a count of a number of periods
15 of a clock occurring within the counted oscillator cycles; the microprocessor unit further comprises a memory accessible to the microprocessor, and wherein the memory comprises encoded software instructions for causing the microprocessor to determine the actual oscillator frequency by dividing the count of the number of oscillator cycles by the count of the number of clock periods; the memory further comprises software instructions for
20 causing the microprocessor to determine an more accurate frequency by combining the counts of a plurality of sampling periods.

In a fifth embodiment, the present invention includes a monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising: a monitoring garment comprising a shirt for the torso of the individual to be monitored, a
25 plurality of sensors, the sensors comprising (i) one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop wherein at least one sensor comprises a transmitter for wirelessly transmitting signals
30 generated by the sensor within the vicinity of the physiological monitoring apparatus, a microprocessor unit comprising (i) a receiver for receiving signals wirelessly transmitted from the sensors, and (ii) a microprocessor for accepting the received signals and for recording digital data derived from the received signals in a removable computer-readable memory media.

35 In first aspects of the fifth embodiment, at least one sensor generates output signals in a digital form, and wherein the transmitter transmits the generated digital signals; the

transmitter and the receiver conform to the Bluetooth standard; at least one sensor generates variable-frequency analog output signals, and wherein the transmitter output is modulated by generated variable-frequency analog signal; all sensors comprise a transmitter for wirelessly transmitting signals generated by the sensor within the vicinity of the
5 physiological monitoring apparatus.

In second aspects, the fifth embodiment further comprises a signal cable, wherein the output of at least one sensor is carried to the microprocessor unit by a signal cable, and wherein the microprocessor records digital data derived from signals carried by the signal cable; the sensors further comprise a cardiac timing sensor for generating signals responsive
10 to occurrence of cardiac ventricular contractions.

In a sixth embodiment, the present invention includes a system for the non-invasive physiological monitoring of physiological parameters of at least one individual comprising: at least one physiological monitoring apparatus comprising a monitoring garment worn on the torso of an individual being monitored, wherein the monitoring apparatus stores in a
15 digital form in a removable computer-readable memory media data, wherein the data is by sensors comprising generated from (i) one or more inductive plethysmographic (IP) sensors flexibly attached to the monitoring garment, and (ii) a cardiac timing sensor for generating signals responsive to cardiac ventricular contractions, and a data repository for reading data from the removable computer-readable memory media that has been recorded by the
20 physiological monitoring apparatus and for storing read data in a data archive, the data repository being remotely located from the physiological monitoring apparatus.

In first aspects of the sixth embodiment, the physiological monitoring apparatus further transmits data wirelessly, and wherein the data repository further receives data wirelessly that has been transmitted by the physiological monitoring apparatus, and then
25 stores the received data; the physiological monitoring apparatus further comprises a microprocessor for processing the generated data for determining physiological events and alarms, and wherein the data wirelessly transmitted comprises the determined physiological events and alarms.

In second aspects, the sixth embodiment further comprises a local data repository
30 co-located with the physiological monitoring apparatus, wherein the local data repository receives data wirelessly transmitted by the physiological monitoring apparatus and stores received data in a local data archive, and wherein the local data repository comprises display terminals for making stored data available to local health care professionals; the data repository further comprises display terminals for making stored data available to health
35 care professionals and to users monitoring the operation of the system.

In third aspects, the sixth embodiment, further comprises a plurality of physiological monitoring apparatus, each apparatus for monitoring a different individual, and wherein the data repository reads data from removable computer-readable memory media recorded by the plurality of physiological monitoring apparatus.

- 5 In a seventh embodiment, the invention further includes a computer readable medium comprising data recorded in digital form, wherein the recorded digital data comprises data responsive with errors of 100 ppm or less to the frequency of an oscillator connected to at least one conductive loop of at least one inductive plethysmographic sensor; and also encoded software for causing microprocessors, data repositories, and the like to
10 perform the described methods.

4. BRIEF DESCRIPTION OF THE FIGURES

- The present invention may be understood more fully by reference to the following detailed description of the preferred embodiment of the present invention,
15 illustrative examples of specific embodiments of the invention and the appended figures in which:

FIG. 1 illustrates a front view of a preferred monitoring apparatus constructed in accordance with to the present invention;

- FIG. 2 illustrates a front view of another exemplary monitoring garment constructed
20 in accordance with to the present invention;

FIG. 3 illustrates a rear view, partly in section, of the monitoring garment of FIG. 2;

FIG. 4 illustrates a front view of a further exemplary embodiment of a monitoring garment;

FIG. 5 illustrates a further view of the monitoring apparatus of FIG. 1;

- 25 FIGS. 6A-C illustrate alternative functional distributions of inductive-plethysmographic signal processing;

FIG. 7 illustrates demodulator processing;

FIGS. 8A-B illustrate alternatives for wireless transmission within an individual's monitoring apparatus;

- 30 FIG. 9 illustrates a particular embodiment of the monitoring apparatus of the present invention; and

FIG. 10 illustrates a system according to the present invention.

5. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

- 35 This section begins with an introductory description of inductive plethysmography, its physiological applications, and its measurement requirements. After the introduction are

detailed descriptions of this invention's practical and effective apparatus for non-invasive, ambulatory monitoring, of pulmonary and cardiac parameters, which in preferred embodiments are various garments incorporating inductive plethysmographic sensors.

5.1. INDUCTIVE PLETHYSMOGRAPH

"Inductive plethysmography" means herein measurement of a cross-sectional area of the body by determining the self-inductance of a flexible conductor closely encircling the area to be measured. Since the inductance of a substantially planar conductive loop is well known to vary as, *inter alia*, the cross-sectional area of the loop, a inductance measurement may be converted into a plethysmographic area determination. Varying loop inductance may be measured by techniques known in the art, such as, *e.g.*, by connecting the loop as the inductance in a variable frequency LC oscillator, the frequency of the oscillator then varying with the cross-sectional area of the loop inductance varies. Oscillator frequency is converted into a digital value, which is then further processed to yield the physiological parameters of interest.

Specifically, a flexible conductor measuring a cross-sectional area of the body is closely looped around the area of the body so that the inductance, and the changes in inductance, being measured results from magnetic flux through the cross-sectional area being measured. The inductance thus depends directly on the cross-sectional area being measured, and not indirectly on an area which changes as a result of the factors changing the measured cross-sectional area.

Various physiological parameters of medical and research interest may be extracted from repetitive measurements of the areas of various cross-sections of the body. For example, pulmonary function parameters, such as respiration volumes and rates and apneas and their types, may be determined from measurements of, at least, a chest transverse cross-sectional area and preferably also and an abdominal transverse cross-sectional area (and optionally further cross-sectional areas). See, *e.g.*, the '872 and '473 Patents; see also, *e.g.*, the '534, '252, '015, 962, '109, '935, and '388, which describe various calibration and processing techniques for respiratory-related inductive plethysmographic signals as well as extensions to measuring intra-pleural pressure and individual lung function, and the description following..

Cardiac parameters, such central venous pressure, left and right ventricular volumes waveforms, and aortic and carotid artery pressure waveforms, may be extracted from repetitive measurements of transverse cross-sectional areas of the neck and of the chest 35 passing through the heart. See, *e.g.*, the '277, '540, '151 Patents. At least, the cross-sectional of a plane at about the position of the xiphoid process is measured. In order to

easily extract cardiac data from variations in these cross-sectional areas, it is helpful to have concurrent measurements of cardiac timing, especially of the onset of left ventricular contraction. Timing measurements are preferably obtained from concurrent ECG measurements, and less preferably from the carotid pulse signal present in the neck. Note:

5 In more detail, area measurements of transverse cross-sectional areas more inferiorly through the heart give stronger indications of left ventricular waveforms, while measurements of areas more superiorly through the heart give stronger indications of right ventricular waveforms. These cardiac signals may be more positively identified by correlation with pulmonary signals. Left ventricular waveforms typically have larger stroke

10 volume on expiration than on inspiration, while right ventricular waveforms typically have the opposite pattern.

Further related parameters may be extracted from these and other signals. From the cardiac-related signals, indications of ischemia may be obtained independently of any ECG changes. Ventricular wall ischemia is known to result in paradoxical wall motion during

15 ventricular contraction (the ischemic segment paradoxically "balloons" outward instead of normally contracting inward). Such paradoxical wall motion, and thus indications of cardiac ischemia, may be extracted from chest transverse cross-section area measurements. Left or right ventricular ischemia may be distinguished where paradoxical motion is seen predominantly in left or right ventricular waveforms, respectively. For another example,

20 observations of the onset of contraction in the left and right ventricles separately may be of use in providing feedback to bi-ventricular cardiac pacing devices. For a further example, pulse oximetry determines hemoglobin saturation by measuring the changing infrared optical properties of a finger. This signal may be disambiguated and combined with pulmonary data to yield improved information concerning lung function. See, *e.g.*, the '425

25 Patent.

Determination of other physiological parameters by measurement of other cross-sectional areas is discussed subsequently.

Useful and effective determination of physiological parameters generally requires inductance measurements of sufficient accuracies at sufficient rates. First, in order to avoid

30 interference using electronics of reasonable cost, it is preferable to measure loop inductance at a frequency which is otherwise relatively unused, or at least not likely to be encountered in most ambulatory settings. The preferred frequency is from about 200 kHz to about 400 kHz which is assigned to aeronautical and aeronautical marine navigation beacons and is below the standard AM broadcast band.

35 Next, necessary measurement accuracies may be determined from known electronic circuit laws combined with measured bodily displacements resulting from the physiological

events being monitored. Measurement accuracies may also be simply determined from observation of particular measurement configuration. Using either approach, it has been determined that respiratory activity generally leads to frequency changes of 500-1000 ppm (parts per million). Cardiac activity generally leads to frequency changes of 50-100 ppm.

5 Therefore, for monitoring both respiratory and cardiac activities, it is most preferably frequency measurements have an accuracy of less than 1-2 ppm, preferably less than 5 ppm, and less preferably less than 10 ppm (and at least less than 100 ppm).

Sufficient measurement rates for respiratory and cardiac activities are generally known in the art and have been confirmed and refined by observation. Generally,

10 respiratory activity is preferably measured at approximately 50 Hz or more; cardiac activity (including cross-sectional areas and any accompanying ECG) preferably at approximately 200 Hz or more, and vascular activity (such as arterial or venous pulsation) preferably at 100 Hz or more.

Of course, particular monitoring tasks may require higher accuracies or rates, or may

15 permit reduced accuracies or rates. Appropriate accuracies and rates may be easily determined by one of skill in the art in view of the monitoring task.

5.2. PREFERRED APPARATUS

In the following, various particular aspects of the present invention are illustrated in

20 various combinations. The illustrated combinations are intended to be exemplary and not to be limiting. One of skill in the art will recognize that these particular aspects, illustrated or not, may be combined in different combinations in order to respond to different monitoring tasks. For a simple example, pulmonary (or cardiac) sensors may be eliminated from a monitoring apparatus where only cardiac (or pulmonary) parameters are of interest. On the

25 other hand, additional sensors may be added to the illustrated embodiments where called for.

5.2.1. MONITORING GARMENT AND SENSORS

Fig. 9 illustrates an embodiment of the monitoring apparatus present invention for

30 monitoring basic pulmonary and cardiac parameters in an ambulatory setting of daily activity with minimum encumbrance to the individual being monitored and in an economical manner. This apparatus includes monitoring garment 1, sensor cabling 2, and microprocessor unit 3.

These components are next described in detail, beginning with Fig. 1. Monitoring

35 garment 1 (Fig. 1) is generally in the form of a sleeveless shirt appropriate for a male of a stout habitus. Modifications of this garment so that it will appropriate for a male with a

trimmer habitus, or for females of various habituses will be readily apparent to one of skill in the art. Alternatively, the garment may be of a cut and material so that, consistent with the requirements to be described, it will be appropriate for individuals of a range of body habituses and possible also for both sexes. In a further alternative, the garment may be
5 made of an elastic material so that a single garment cut and size is able to fit a wide variety of individuals.

To measure basic pulmonary parameters, garment 1 is equipped with chest inductive plethysmographic sensor band 4 and abdominal inductive plethysmographic sensor band 6. Band 4 is preferably positioned just inferior to the axilla, and band 5 is preferably
10 positioned 1 to 2 cm superior to the umbilicus. To measure basic cardiac parameters, garment 1 is equipped with thoracic inductive plethysmographic sensor band 5 and optional neck inductive plethysmographic sensor band 7, which is separate from garment 1. Band 5 is preferably positioned at the level of the xiphoid process, and band 7 is preferably
15 positioned 1 to 2 cm superior to the base of the neck. Preferably, the garment has cutouts 12 for attaching ECG electrodes and ECG leads 10 (approximating right arm signals) and 11 (approximating left arm signals) and 12 (approximating left leg signals). In alternative embodiments, sensor bands 4 and 6 or sensor band 5 may be eliminated, or optional sensor band 7 may not be present, or additional sensor bands may be present, or so forth.

In this embodiment, signals from all the sensors mounted on or with the garment,
20 including any ECG signals, are conducted to external microprocessor unit 3 via signal cable 2. Signal cable 2 is extended by optional signal cable 13 to conduct signals from optional neck band 7. Connections between signal cable 2 and the various sensors reside underneath openable flap 18, which both protects the connections and any electronic modules closely associated with the sensors from disruption or damage and also presents a more aesthetic
25 surface appearance. In alternative embodiments, the signal cable may be attached to the garment by several snaps positioned or the like, associated electronic modules may be carried in pockets of the garment, and connections firmly held in place by mechanical means. Other manners for protectively securing the signal cable will be apparent to one of skill in the art and are within the scope of the present invention.

Also underneath the flap is a zipper, preferably the full length of the garment, to
30 assist in placing and removing the garment. Alternatively, and less preferably, the garment may be arranged for placement and removal over the head in one piece. In this case, there is no need for a zipper or other fastening devices. Other similar garment fastening devices may be used, for example, a plurality of buttons and buttonholes, or a plurality of loops
35 fitting into grommets, or a plurality of ties, or a zipper-like device with halves fitting

together continuously instead of by a plurality of teeth. More than one zipper may also be used.

Inductive plethysmographic (IP) sensor bands 4, 5, 6, and 7 and the garment 1 itself preferably include several functional or structural elements to meet several conditions advantageous for good signal quality. First, the sensor bands include the sensors themselves, which are conductor loops around the body in the substantially transverse planes whose cross-sectional area is to be measured. Since the bands should remain in close circumferential contact to the torso (of the body of the individual to be monitored) in order to accurately sense cross-section areas that change during activity, the conductor should be longitudinally flexible to accommodate normal physiological movements. Further, to achieve sufficient measurement accuracy and rate, the LC circuit containing the conductor loop must have a sufficiently high Q factor. Generally, at the preferred frequencies, the conductors preferably have a resistance of less than approximately 1 Ω (Ohm). Any flexible low-resistance conductor may be used. In preferred embodiments, the conductor is metallic wire, sinusoidally arranged for expansion and contraction without hindrance of the cross sectional area to inductance relationship. For example, the sinusoidal wire arrangement may have an "amplitude" of 1 to 2 cm with "wavelength" adequate to provide for longitudinal flexibility sufficient to accommodate anticipated activity.

For chest sensor band 4 and abdominal sensor band 6, which obtain respiratory signals, generally one loop of conductor about the body achieves sufficient signal quality. For thoracic sensor band 5, and also for neck sensor band 7, several loops, generally from 2 to 4 loops, achieve sufficient signal quality.

Next, in order to remain in close circumferential contact to the body, it is preferable that the sensor bands also include an elastic material providing longitudinal elasticity sufficient to retain the band against the body. For example, the bands may include a longitudinally arranged strip of elastic material similar to that in elastic bandages well known in the art. The sensor conductors may be attached by a plurality of distinct connections, which may be sewn loops of thread. More preferably, the bands may include a woven or knitted elastic material into which sinusoidally arranged copper wire is integrally included during the weaving or knitting process. See, *e.g.*, U.S. patent application serial no. 09/774,333, filed Jan. 31, 2001, and assigned to the assignee of the present application (included by reference herein in its entirety for all purposes). The sensor conductor may be attached to underlying material of other ways known in the art, for example, by glue.

In addition to simply remaining in close circumferential contact with the body, the bands should not move inferiorly or superiorly (collectively, longitudinally) over the surface of the torso of body, in other words, be longitudinally stable, during normal daily activities.

This is advantageous so that signals from each band are responsive only to the one intended cross-sectional area. For a sufficiently trim male engaging only in light activity, the elasticity that keeps bands in close circumferential contact may be sufficient to achieve longitudinal stability. However, it has been discovered that generally, and especially for
5 normal or vigorous activity, such as jogging or other athletic activities, or for individuals with a larger body habitus, this circumferential elasticity may not be sufficient for longitudinal stability.

Therefore, in the embodiment illustrated in Fig. 1, each band is also equipped with an individual tightening device 8, which permits individual adjustment of the tightness of
10 each band as needed. One such tightening means, schematically illustrated by tightening device 8, is a gripping device in which metal or plastic teeth or paired rings grip excess material 8 attached to the band. Pulling excess material so that the gripping device holds the excess material under tension will tighten a band, while release of the mechanism (*e.g.*, rings or teeth) of the gripping device loosens the bands for undressing. In this manner, after
15 dressing in the monitoring garment, the bands can be individually adjusted to a tightness discovered to be sufficient after an initial period of monitoring garment use. The bands may be loosened or released in preparation for undressing. Preferably, the "excess" material and gripping device form a cinch (also known as a girth) in that the "excess" material is part of a band of material circling the patient included as part of the inductive plethysmographic
20 sensor band assembly. Alternative tightening means include belt-buckle-like arrangements with a tooth for protruding through one of plurality of holes in the excess material, or a slidable spring-loaded device that grips the excess material against a grommet as used in a drawstring, or simply a bow-type knot used like a shoe lace, or other gripping means. Any cinch or girth material must of course also be elastic, but preferably stiffer (less elastic) than
25 existing sensor band material.

One aspect of longitudinal stability is that during activity the garment material itself may pull on the bands causing mechanical coupling both of a band sensor to a more extended longitudinal region than intended and also between adjacent, separate band sensors. This coupling may mix decrease signal specificity, and may mix together signals
30 from the so-coupled sensors leading to decreased accuracy of physiological parameter determination, or even an inability to determine certain parameters. Therefore, it may be advantageous for the garment material between the sensor bands not to be tight, but rather have an excess sufficient to accommodate longitudinal stretching and other longitudinal motions that accompany activities of all expected degrees of strenuousness. (For example,
35 such excess material may be present in regions 19 of garment 1 of Fig. 1.) Alternatively, the garment material may be sufficiently stretchable in a longitudinal and the bands

sufficiently elastic to be circumferentially tight, or synched to be sufficiently tight, so that longitudinal motions are accommodated mainly by stretching of the garment with little or no longitudinal band movement with respect to the torso. These designs may be combined so that the garment between the bands has some excess of an elastic material. In particular, 5 where the garment is made of an elastic material to accommodate a range of body types, care must be taken to prevent longitudinal mechanical coupling occurring, especially for individuals of larger body types relative to the garment size and cut.

Where the garment is zippered, or otherwise similarly fastened, at least some of the inductive plethysmographic (IP) sensor bands are necessarily interrupted. However, the 10 garment fasteners should be arranged such that, when the garment is fastened, circumferential band elasticity is established even though elastic in the sensor bands is necessarily interrupted at the garment division. With zippers, this is easily achieved because of the substantially continuous nature of a zipper fastener. Further any band tightening devices must also cooperate with the garment fasteners. With a cinch (or girth) 15 held by a gripping device, this is easily achieved by allowing excess cinch to extend across the division in the garment. Lastly, the conductive loop is interrupted at the garment division, and may bridge this division by equipping ends of the loop with mating pair of a plug and a connector. Alternatively, one or both ends of the conductor bands may plug into connectors carried on the signal cable (see below). One of skill in the art will readily be 20 able to similarly arrange the IP sensor bands for cooperation with other types of garment fasteners.

In addition to the sensors already described, additional sensors may be incorporated with monitoring apparatus of the present invention. For correctly interpreting physiological parameters obtained during ambulatory monitoring, it is advantageous to have information 25 from which the posture of the patient can be determined. At least, it is useful to determine if the monitored individual is lying, sitting, or standing, and if standing, whether still, walking, or running. In a preferred embodiment, this information is provided by accelerometers that measure orientation with respect to gravity. The apparatus illustrated in Fig.1 preferably includes an accelerometer attached to garment 1, optionally by being 30 integrated into an electronic module associated with one of the band sensors. Such a single sensor can provide only the orientation of the torso. Further information may be provided by optional accelerometers strapped to one or both thighs. Signals from these additional accelerometers may be conducted to signal cable 2 by means of secondary cables 14, which attach to the signal cable at connector 15.

35 Further, any transdermal sensor may be incorporated into the illustrated monitoring apparatus. Such sensors may include pulse oximeters, which measure hemoglobin

saturation in a finger, blood pressure sensors of various types, transdermal sensors indicative of blood chemistry, for example, blood glucose sensors or sweat electrolyte sensors, and so forth. Signals from these sensors may reach microprocessor module 17 over signal cable 16. Preferably, these sensors will present a standard interface to the
5 microprocessor module, for example an RS-232 or more modern serial interface. Further, it may be advantageous to obtain more complete ECG information, such as by receiving signals from 7 or 12 leads placed in manners well known in the art. A further additional sensor may be a throat microphone, which is useful for detecting snoring during sleep and talking during wakefulness. Detection of snoring during sleep is a valuable indication of
10 incipient or actual upper airway obstruction useful in sleep apnea studies. In such an embodiment, the microprocessor module may accumulate information reflecting a broad array of transdermally measurable physiological parameters in a scandalized ???manner and in a standardized format.

15 **5.2.2. MICROPROCESSOR UNIT AND CABLE**

As described previously, the monitoring apparatus of this invention may be provided with primary and secondary signal cables. Fig. 1 illustrates primary signal cable 2 which carries signals from the primary sensor bands 4, 5, and 6, and secondary sensor band 7. This cable also has provision for carrying ECG signals, provided, for example, over leads
20 10 and 11, and provision for signals from other sensors received at connector 15. Further, secondary signal cable 16 may optionally carry signals from a number of other sensors arranged on the body.

Signals gathered by the monitoring apparatus are received by microprocessor unit 3. Unit 3 performs at least basic data entry and storage functions, and optionally performs
25 alarm functions, communication functions, and power management functions. The unit may be built as an add-on to existing personal digital assistants (PDAs), cell phones, cell phone/PDA combinations, bidirectional pagers, especially those used for e-mail exchange, and other similar handheld devices. Also the unit may be a custom design including at least a microprocessor and associated components, and optionally signal processor circuits.
30 Preferably, unit 3 has display screen 17 which is touch sensitive for data input by the monitored individual. Other user interface features may be provided, such as voice command recognition, voice or audible alarm output, attachable keyboard, and so forth. This unit may also optionally include wireless communication circuits. Also, although Fig. 1 illustrated unit 3 as possibly hand-held, it may also be carried on an individual normal
35 clothing, for example, on a belt, or may be placed in a pocket provided on garment 1.

A first data entry function is to receive and store information input by a monitored individual. For example, a monitored individual may enter major activities during the day along with any symptoms that might be observed.

A second data entry and storage function, to receive and store digitized signals generated by the sensors of a monitoring apparatus of this invention, is closely linked with possible communication functions. Preferably, the present invention conforms to well known standards for "standard event recording" by advantageously and preferably storing all raw signal data, whether or not it is used only in summary form for health care personnel. Storing raw data in a central repository is encouraged by regulatory authorities, and is important in quality control of monitoring by the present invention. Further, health care personnel may from time-to-time wish to examine the raw data indicative of the physiological events occurring in the monitored individual, which is possible by accessing the central repository.

However, this raw data may be voluminous, even for a basic monitoring garment. Table I presents the data rates generated by the apparatus of Fig. 1 wherein operation is with preferred sample precision and data rate for each sensor.

Table I - Exemplary Data Rates

Sensor	Bits per sample	Samples per second	Data rate (MB/hr)
Chest sensor band	16	50	0.36
Abdominal sensor band	16	50	0.36
Thoracic cardiac sensor band	16	200	1.44
Neck sensor band	16	100	0.72
Accelerometer	8	10	0.04
ECG	12	200	1.08
Pulse oximeter	8	50	0.18
Throat microphone	8	10	0.04
TOTAL			4.22 (= 1.2 kbits/sec)

Therefore, the present invention includes various tradeoffs for the storage or raw data, which depend primarily on available battery power and accessible wireless facilities. For example, if high bandwidth wireless data transfer, for example 64 kbits/sec or greater, is available throughout an individual's daily activities, currently (as of this application's filing

date) an unusual possibility, then wireless transmission of all raw data would require an apparatus transmitter duty cycle of 2% or less, which may be acceptable in view of available device battery power and the cost of wireless access. On the other hand, wireless access currently available supports data transmission rates at best of 14.4 kbits/sec. At these rates
5 the apparatus transmitter would have a virtually 100% duty cycle, which is likely to be an unacceptable power and wireless access cost.

Alternatively, data may be stored locally in microprocessor unit 3 and transmitted periodically only in bulk. For example, a commonly available 64 MB flash memory module may easily store raw data for 12 or more hours. A 128 MB module could hold 24 hours of
10 data. A full flash memory module may be replaced and sent by overnight mail services to the central repository. Alternatively, the data may be sent by high-speed wired digital connection (e.g., DSL or cable modem to internet) directly to the central repository. Other removable memory technologies, for example, micro-hard-drives or micro ZIP drives, may also be used. In this embodiment, unit 3 may communicate wirelessly only important or
15 significant physiological events, such as alarms recognized. This would be a much smaller amount of data easily transmitted currently in most locations.

Therefore, depending on available wireless data rates and access cost, on available apparatus battery power, and available removable memory capacities, the present invention includes microprocessor unit designs from wireless transmission of all raw data to a central
20 repository to local storage of all raw data with periodic transmission. The latter embodiment is currently preferred for most locations.

A further data storage embodiment includes local, private wireless transmission of data from a microprocessor unit of a monitoring garment to a local receiver within a short distance, perhaps 10 to 1000 feet, of the individual being monitored. From the local
25 receiver, the data may be stored at a local computer system, for example a PC-type computer, for periodic transmission to a central repository for access by health care providers. The periodic transmission may be via removable media a few times daily, as described above, or may be via standard or high speed access (DSL or cable modem) perhaps hourly. Alternatively, the central repository may be replaced by a local server PC-
30 type computer accessed by health care providers. Although not changing transmitter duty cycles, local wireless transmission would eliminate access costs to public wireless facilities and may be of higher speed, and therefore, make wireless transmission of raw data a more attractive tradeoff. The embodiment would be appropriate for an individual ambulatory yet confined to a residence, or health care facility, or relatively small workplace. It may not be
35 appropriate for individuals engaging in their normal daily activities.

In an alternative embodiment, the microprocessor may compress the signal data prior to storage. This compression may be implemented in software encoding an appropriate known compression technique. An exemplary technique is to subtract a base carrier frequency from each frequency datum so that recorded frequency data are offset to have a substantially zero average frequency. Further, each offset frequency datum may be recorded as a difference with respect to one or more previous offset frequency data. Periodically, the current carrier frequency and a offset frequency datum may be recorded to synchronize decompression software.

Next, microprocessor unit 3 may optionally recognize alarm conditions and generation alarm signals, which are in all cases audible but may also involve screen-display of visual information. Alarm conditions recognizable by a microprocessor unit of average capabilities are of two basic types. The first type is a discrete temporal event. For example, heart rate increases suddenly to dangerous levels, or paradoxical wall motion is observed, or breathing slows or ceases dangerously. The second type of alarm condition is a trend progressing over one to a few hours. For example, in a congestive heart failure patient, over two hours an increasing respiratory rate, perhaps coupled with sustained cardiac rate changes, may signal early the onset of pulmonary edema. Similar changes may be alarmed for individuals at high altitude to warn early of dangerous forms of mountain sickness. Other discrete and trends that are known to those of skill in the art may also be alarmed. Alternatively, more powerful microprocessor units may recognize more complex alarm conditions, perhaps by employing artificial techniques such as neural networks or rule-based systems.

Finally, power management is an important optional function which may be performed in the microprocessor unit. Preferably, the various electronic modules and sensors that are included in a monitoring apparatus according to the present invention include power management facilities that provide reduced power consumption whenever a module or sensor is not in active use. For example, reduced power consumption may be commanded by control signals generated by the microprocessor unit. Additionally, battery power may be physically a part of unit 3. Alternatively, a separate battery unit could be arranged, for example, in a pocket of garment 1.

5.2.3. FURTHER MONITORING GARMENT EMBODIMENTS

Referring now to Figs. 2 and 3, further embodiments of the non-invasive physiologic monitoring garment 30 comprise inductive plethysmographic sensors 20-25 which are embroidered, sewn, embedded, woven, printed onto or otherwise affixed to or carried on a garment 31 that is worn over and about the torso of an individual to be monitored. As

shown in Figs. 2 and 3, the garment may comprise a turtle-neck long-sleeved garment 31 including portions covering the pelvic region and upper thighs. Instead of the long-sleeved garment 31, the garment in a further embodiment may comprise sleeveless shirt 31a shown in Fig. 4, which does not include the sensors 25. Furthermore, the garment may also be
5 made without the leg portions shown in Fig. 2. Monitoring garment 30 further includes electrocardiographic electrode sensors 26 that may be sewn, embedded, or fixed with adhesive or the like to the inside of the rear face of the garment 31. Alternatively, the electrocardiographic electrode sensors 26 may be directly affixed to the individual to be monitored. The garment 31 may be fastened snugly onto the body using fastening devices
10 32 such, for example, as velcro strips or ties 16 (see Figs. 2 and 3). Alternatively, the garment may comprise a shirt without fastening devices as in Fig. 4. In this embodiment, the sensors 20-25 may include an elastic material portion for holding them in place on the individual's torso.

Microprocessor unit 33 comprises, in one embodiment, an oscillator-demodulator
15 (see below) unit for the inductive plethysmographic sensors 20-25, and either has multiplex capabilities or takes the form of a similarity of separate oscillator modules tuned to respectively different frequencies for eliminating cross-talk between the various sensors 20-25. Oscillator modules 20a-24a may also be attached directly to respective inductive plethysmographic sensors 20-24 (see Fig. 4). Alternatively, the oscillator modules may be
20 located directly in the microprocessor unit 33. The microprocessor unit is shown attached to a side of the garment at the waist of the individual to be monitored; it may, however, alternately be attached or carried in any comfortable position or location on or about the body of the individual. As described above, microprocessor unit 33 comprises a recording/alarm unit which collects the monitored signals from the sensors 20-26.
25 Furthermore, microprocessor unit 33 includes a processor for, *inter alia*, determining alarm conditions and providing data logging functions. Microprocessor unit 33 may also include an output device 45 such, for example, as a sound system optionally with a visual display, for providing alarms and action recommendations to the individual and/or healthcare provider in a preferably area. In an embodiment, the sound system provides these alarms
30 and action recommendations as plainspoken audible statements.

In addition to a sound system that reproduces audible messages, the output device 45 may be a display unit such as a monitor screen that displays the messages. This alternative may for example be used when the individual to be monitored is deaf or hard of hearing, or where the message contains a great deal of information which may be difficult to
35 comprehend or appreciate when merely listening to an audible message. Such a modification also requires an additional signal to be directed to the individual being

monitored when a new message is present, since the individual may not be within a line of sight of the monitor screen of the microprocessor unit 33. For this purpose, microprocessor unit 33 may include or activate a signaling device such as a illuminatable lamp for informing the individual being monitored that there is a new message. Since microprocessor unit 33 is
5 mounted on the monitoring garment 30, the signaling device may also when activated effect a vibration of the microprocessor unit 33 which will be felt by the individual being monitored.

Microprocessor unit 33 may be built to include a Personal Digital Assistant (PDA) such as a HandSpring or Palm Pilot or any mobile device capable of wireless
10 communication. In a preferred embodiment, electrodes 20-26 are wired to an electronic module which may be plugged into the microprocessor unit 33. The module uses the processor of the microprocessor unit to perform monitoring, alarming and data logging functions. Furthermore, the monitored signals may be compared to default values to ensure that they are within an acceptable range. If the monitored signal exceeds or falls below the
15 acceptable range of values, the alarm function alerts the individual.

The microprocessor unit may further include input capabilities so that the individual can input information such as symptoms, activities, medications that have been taken, and mood. These input capabilities may be menu driven such that the individual merely selects from a list. Alternatively, the individual may input his symptoms by typing on a keyboard
20 or writing on a touch sensitive screen.

The microprocessor unit 33 may also be connected or linked to a receiving unit 34 located at a separate or remote site that is attended by health care providers for transmitting the data received from the monitoring garment 30, and associated alarms and/or messages, to receiving unit 34 so that the health care providers at the remote site may view and
25 analyze the data. Furthermore, the individual may then use the input capabilities to inform the health care professional regarding the symptoms, activities, medications, and mood. The transmission to the remote site may be made via a modem, Internet connection, satellite hookup, cable, or any other communication system or arrangement, such a standard wireless telephone networks. The connection between microprocessor unit 33 and receiving unit 34
30 may also allow health care providers at the remote site to return information to the microprocessor 33. For example, the health care providers may wish to provide specific instructions to the individual being monitored. In addition, the PDA may log the data received from the monitoring garment 30 to a local or remote database for tracking the condition of the individual and for comparison to other individuals. This enables continued
35 modification and refinement of the diagnostic algorithm in the module or the

microprocessor unit 33 and transmission of action recommendations from the receiving unit 34 to the microprocessor unit.

The monitoring garment 30 or 30a may transmit data to the receiving unit at a rate ranging from approximately 1-1000 points/second (depending on the available sensors).

- 5 Optionally, a one minute trend numerical value reduced from data processed waveforms is transmitted every five to ten minutes in which case the monitoring garment may transmit data at the rate ranging from approximately 1-1000 points/second only when an adverse or preprogrammed event occurs to thereby conserve the batteries powering the microprocessor unit 33 on the monitoring garment.
- 10 The structure and operative functionality of the individual sensors 20-26 will now be explained in further detail. A neck inductive plethysmographic sensor 24 is sewn, embroidered, or embedded, for example, to the area of garment 31 or 31a. Sensor 24 monitors jugular venous pulse, carotid arterial pulse, intrapleural pressure swings related to respiration, contraction of neck muscles, and swallowing deflections. Estimations of the
- 15 central venous pressure from the data collected by sensor 24 compares well to values simultaneously recorded using intravascular catheters. Since the jugular venous pulse depicts an 'a' wave related to atrial contraction, which is a substitute for the 'P' wave of the electrocardiogram, data from sensor 24 may aid in differentiating arrhythmias and supraventricular tachycardia with aberrant ventricular conduction from ventricular
- 20 tachycardia. The recording of the arterial pulse in conjunction with an electrocardiograph allows computation of the systolic time intervals which may be used for estimating the mechanical function of the left ventricle. Sensor 24 may also record swallowing deflections as sharp, transient waveforms superimposed upon slower respiratory deflections and vascular pulses.
- 25 An abdominal plethysmographic sensor 20 and a rib cage plethysmographic sensor 21 are sewn, embroidered, or embedded, for example, in the abdominal and rib cage portions of garment 31 or 31a for monitoring the expansion and contraction of the abdomen and rib cage, respectively. Sensors 20 and 21, used together, are referred to as a respiratory inductive plethysmograph and are employed for recording breathing patterns.
- 30 A thoracic inductive plethysmograph sensor 22 is sewn, embroidered, or embedded, for example, into garment 31 or 31a around the xiphoid process region. Sensor 22 may be formed of one or more plethysmographic coil-type sensors and operatively monitors the beat by beat ventricular volume during breath holding and during slow breathing. Analysis of the resulting waveforms by the microprocessor unit recording/alarm unit 40 enables
- 35 computation of changes in cardiac output and stroke volume and of parameters related to systolic and diastolic functions. Analysis of a derivative of the ventricular waveforms

yields parameters analogous to Echo-Doppler measurements of the mitral valve. The deceleration time of the mitral flow velocity parameter can provide an estimate of pulmonary capillary wedge pressure in individuals with compromised left ventricular function. Longer deceleration times are consistent with normal and shorter times with
5 elevated pulmonary capillary wedge pressures.

Two hemithoracic inductive plethysmographic sensors 23 are sewn, embroidered, or embedded, for example, into garment 31 or 31a on the right and left sides of the upper thorax. These sensors 23 enable measurement of inequalities in regional expansion with breathing and paradoxical motion between the two hemithoraces. Such inequalities suggest
10 pleural effusion, diaphragmatic hemiparesis, or pneumothorax and may aid in diagnosis of certain clinical circumstances.

Limb inductive plethysmographic sensors 25 are sewn, embroidered, or embedded, for example, at the elbow and wrist areas of the garment 31. Sensors 25 record vascular pulses over the vessels of the limb or extremity about which it is placed. Sensors 25 may be
15 used to record peripheral blood flow using standard plethysmographic occlusion techniques, pulse transit time by using a pair of separated sensors 25 on the extremity, or pulse transit time from arterial pulse in the neck to the extremity. Sensors 25 may also provide wide-band external pulse recording of systematic blood pressure during cuff deflation.

The preferred embodiment of monitoring garment 30 further includes
20 electrocardiogram (ECG) electrode sensors 26 (Fig. 3). As stated above, the ECG electrode sensor may be mounted on the monitoring garment 30 or, alternatively, may be directly applied to the individuals body and connected to the PDA 33 via a wire.

The combination of RR intervals of the ECG measurements from sensors 26 and the tidal breath waveform from the respiratory inductive plethysmographic sensors 20 and 21 as
25 described above may be used to determine respiratory sinus arrhythmia which is a measure of autonomic nervous system function. High values of this measure signify predominant parasympathetic nervous system activity and low values predominant sympathetic nervous system activity.

A body position sensor 27 may also be sewn, embroidered, or embedded, for
30 example, in garment 31 or 31a to indicate the individual's posture. Body position sensor 27 may comprise one or more standard available accelerometers.

Finally, pulse oximeter sensor 28 (Fig. 3) may also be used in conjunction with the monitoring garment 30 or 30a. Pulse oximeter sensor 28 is generally placed at a distal fingertip of the individual or subject to measure arterial oxygen saturation and body
35 movements. Although pulse oximeter 28 need not be carried on or as a direct component of the monitoring garment 30, detected information from oximeter 28 may be treated in a

manner similar to data from sensors 20-26 by microprocessor unit 33. True values of arterial oxygen saturation are thereby distinguishable from values affected by motion artifacts using appropriate software algorithms.

5 The recording/alarm functions of the microprocessor unit 33 operatively provides, by way of illustrative example, the following functionality:

 (1) messages assuring proper functioning of the monitor, such for example, as “system operating properly”;

 (2) messages concerning actions to be taken in the event of malfunction, such, for
10 example, as “system not operating properly, make sure the disk is inserted properly”, or “system malfunction, contact the equipment manufacturer” (the name and address may also be supplied);

 (3) messages concerning correct or incorrect placement and detachment of sensors 20-26 and their lead wires;

15 (4) messages relating to vital signs information, significance, and actions to be taken by the individual in response thereto;

 (5) periodic messages concerning the stability of vital signs at preselected intervals or upon request of the individual or health care provider for assurance purposes, such for example, as “it is now 10AM and there has been no change in the vital signs”;

20 (6) messages relating to specialized physiologic parameters information, significance, and recommended actions in response thereto;

 (7) directions including instructions entered by an attending health care provider, and

 (8) reminders directing the individual to take medications (the recording unit may
25 log compliance by monitoring when the individual swallows if the medication is to be taken orally, or monitoring breathing patterns if the medication is to be taken in aerosol form).

 In addition to providing such messages, the recording/alarm function may monitor the individual for effectiveness and proper functioning of assisted ventilatory and continuous positive air pressure (CPAP) devices. The recording/alarm function also logs
30 data into a database as physiologic waveforms for one-minute numerical trends which may be transmitted to the remote receiving unit 34 automatically or upon receipt of a request for review by the provider at the remote receiving unit 34.

 Instead of concurrently collecting data from all of the sensors and detectors of the monitoring garment 30 or 30a the types of physiologic parameters to be monitored may be
35 limited as a function of the specific condition of the individual. Furthermore, garment 31 may comprise any combination of sleeves, turtle neck, and leggings as required by the

specific condition of the individual. For example, if an individual has asthma, pertinent parameters such as respiratory drive/ventilation (peak inspiratory flow/ventilation and/or peak inspiratory acceleration/ventilation) should be monitored closely as non-invasive parameters of increasing bronchospasm above a predetermined threshold. This measure
5 will be utilized to provide directions to the monitored individual via output device 45, such for example, as "you have signs of bronchospasm; please take your aerosol medication now!" If aerosol medication is taken correctly and the proper breath holding pattern is observed by the microprocessor unit 33, then output device may state, "aerosol taken, good!" If after 30 minutes, there is no improvement or there is a worsening of specific
10 measures and/or vital signs, the microprocessor unit 33 may state, "call your doctor immediately!" or "get transportation and go immediately to the emergency room."

As another specific example, if the individual has chronic heart failure, then the deceleration time from the derivative of the left ventricular volume curve obtained with the thoracocardiograph, the central venous pressure and respiratory sinus arrhythmia should be
15 closely monitored. The deceleration time has been found to be the most predictive parameter that hospital admission is needed for treatment of chronic heart failure. In one study, values below 125 msec were the threshold associated with required hospital admission. Thresholds may be programmed into microprocessor unit 33 that instructions are delivered to the individual being monitored before the 125 msec level is reached. For
20 example, if the baseline deceleration time of 160 msec falls to 140 msec, then microprocessor unit 33 may state, "take an additional diuretic tablet today at 5 PM." If the deceleration time falls to 120 msec, the microprocessor unit may state, "call your physician immediately." Central venous pressure reflects fluid balance in the body; low values indicate hypovolemia as might take place with overdosing with diuretics, and high values
25 with increasing severity of heart failure. Thus, if CVP on one day is 8cm H₂O and the following day is 4 cm H₂O, the microprocessor unit might state "call your doctor immediately for advice on your medications."

With regard to the monitoring of medicine taking compliance, the desired times of day for taking medications are programmed into microprocessor unit 33. At appropriate
30 times, the unit may state "take one capsule of #1 - or one capsule of verapamid now!" Microprocessor unit 33 may also include input devices such as, for example, a bar code reader or other reader so that when the individual takes out a medication vial with a bar code, information from the bar code is passed to the optional bar code reader. Alternately, the individual may enter information on the medications using a manual input device such
35 as, for example, a keyboard or a simple array of buttons as stated above. By clicking or pressing one of the buttons, the individual being monitored manually updates the

recording/alarm device to indicate compliance with the scheduled medicine. As mentioned above, when the individual takes medication, swallows may be logged from the neck inductive plethysmograph waveform, thereby suggesting compliance. After the taking of medication, the individual may pass the vial over the optical reader or activate a switch to
5 create window timing marks in the data stream that can be analyzed and stored in microprocessor unit 33 and/or receiving unit 34.

The physiologic parameters may also be programmed for monitoring effectiveness based upon CPAP or BiPAP ventilatory requirements. Nocturnal CPAP and BiPAP are often used for treatment of the obstructive sleep apnea syndrome, which is marked by
10 apneas and increases in upper airway inspiratory resistance. The ratio of peak inspiratory flow to mean inspiratory flow (PIF/MIF), derived from the tidal volume waveform of the respiratory inductive plethysmograph 20 and 21, provides a numerical value for the shape of the inspiratory flow curve. An unobstructed inspiratory flow contour has a sinusoidal shape and the value of this parameter, PIF/MIF , is $\pi/2 = 1.57$. As an inspiratory obstruction
15 develops, the inspiratory flow waveform becomes flattened and approaches a PIF/MIF value of 1.0. Significant flattening begins with a threshold value at or below 1.3. In some instances, inspiratory obstruction is marked by a brief prominent spike near beginning inspiration that gives PIF/MIF values of approximately 1.85 or greater. Therefore, optimum CPAP should provide values ranging from 1.3 to 1.85. If PIF/MF is found to be equivalent
20 to 1.2 for a predetermined period of time, then the recording/alarm unit may deliver a message to the individual or to the personal health care provider, with increasing decibels of sound until the problem is corrected, stating "increase CPAP 3 cm water pressure now". Algorithms have been described for automatic adjustment of the level of CPAP pressure based upon indices related to the shape of the inspiratory flow curve.

25 Since CPAP is generally administered using a nasal mask, it is subject to leaks, particularly at the mask-skin interface. Leaks can be discerned by the recording/alarm unit 34 by comparing the tidal volumes between the values delivered from the CPAP apparatus and those received by the individual. The latter is obtained with respiratory inductive plethysmography using the sensors 20 and 21. For example, if the inspiratory volume per
30 breath from respiratory inductive plethysmography sensors 20 and 21 was found to be 200 ml and the volume delivered by the CPAP device is 500 ml, then a leak in the CPAP system of 300 ml is indicated and the recording/alarm unit may state "wake up and adjust your mask, it is leaking." Mask leaks are also a problem in administering ventilatory support to individuals with respiratory failure or respiratory muscle weakness. Monitoring of volumes
35 delivered versus volumes received is effective in diagnosing such leaks.

5.2.4. FURTHER ELECTRONICS EMBODIMENTS

The present invention includes several possible distributions of electronic circuitry between electronic modules carried on garment 1, which are associated with sensors on this garment, and unit 3. Fig. 5 illustrates options for the physical distribution of circuitry, primarily circuitry for processing inductive plethysmographic signals. Here, as before garment 1 includes inductive plethysmographic sensor bands 4, 5, and 6 which are connected to microprocessor unit 3 by primary signal cable 2. Also present at ECG leads 10 and 11 along with a third lead 47 arranged underneath garment 1. Together these leads collect signals representative of a standard 3-lead ECG.

Fig. 5 principally illustrates electronic modules 42, 43, and 44 connected as at 45, to the flexible conductors in the sensor bands, and physically close to the sensors. When the garment is zippered, or otherwise fastened, these modules may support connectors for bridging the conductive loops across the garment division. These modules are disposed underneath flap 18, which is here illustrated as opened, for example when a monitored individual is in the process of "wiring-up" during dressing (or undressing). This flap may be held normally closed by, for example, velcro strips. Also illustrated is fastener 41 (partially hidden by the extension of signal cable 2), such as a zipper, which facilitates dressing by opening the garment. Also facilitating dressing, flexible inductive plethysmographic conductors may plug and unplug into the local modules. Alternatively, for simplicity, the ECG leads may be permanently attached, or they may also plug and unplug from the modules.

The local electronics modules optionally contain circuitry for initial processing of the inductive plethysmographic signals, while circuitry for final processing is carried in unit 3. These modules may also contain initial processing circuitry for other sensors. For example, modules 42 and 43 may contain ECG circuitry, perhaps simply analog pre-amplification and filtering or perhaps also A/D conversion.

Preferably, these electronics modules are permanently attached to the signal cable to minimize the number of parts to be handled during wiring up. Alternatively, these modules may be retained in pockets in the monitoring garment, and plugged and unplugged from the signal cable during wiring up.

Next, Figs. 6 and 7 illustrate possible functional distributions of electronic circuitry for processing the plethysmographic signals. With respect to Figs. 6A-C, the functions of the osc (oscillator) block(s), the demod (demodulator) block(s) and the microp (microprocessor) block are substantially the same and will be described once with respect to Fig. 6A. First illustrated are three conductive loops coupled to a single or to individual oscillators. The oscillators are LC oscillators with a frequency responsive to variations in

the loop inductance, and may be of any type known in the art. They preferably are of very low drift due to temperature and other compensations.

Preferably, the loops are coupled to the oscillator(s) through an impedance step-up transformer which steps-up the loop impedance so that reasonable values of capacitor C (for example, greater than 1,000 pF) may be used leading to stable oscillator function. The impedance step-up also multiplies loop-inductance variations leading to a greater signal range. Further, the transformer provides an uninterrupted loop circuit isolated from the powered electronic circuitry. This isolation improves individual safety. Loop isolation is also improved by slightly offsetting the resonant frequency of each loop, for example, by 10 to 50 kHz. Finally, it has been found important that the total loop resistance be low, no more than approximately 1 Ω , to achieve a high Q factor.

The demod blocks demodulate the variable frequency oscillator signal in the sense that they measure the frequency and provide digital data to the microp block. Their function is further described with reference to Fig. 7.

The microp block includes a microprocessor programmed to perform, *inter alia*, the functions described above including the basic data entry, storage, and communication function. This block may be based on a commercially available PDA-like device, or may be custom designed. In either case, it will be understood to include a microprocessor and supporting components, typically including RAM and ROM memory, a display interface, user input-output interfaces, a communications interface or an interface to removable media, and so forth. The memories will be loaded with programs translated from any convenient programming language.

In view of this general description, Fig. 6A illustrates an embodiment with a single instance of all functional blocks switched between and shared by the three inductive plethysmographic loops. Here, local modules 42, 43, and 44 would include little more than connectors from the conductive loops for bridging any division in the garment and to signal leads to the electronic functional blocks housed in unit 3. As discussed, the entire path between and including the conductive loops and the step-up transformer in the osc block preferably has a resistance of less than 1 Ω . Therefore, the switch SW is preferably a low resistance controllable switch for analog radio-frequency signals. Such a switch is currently available from Dallas Semiconductor/Maxim. Where such a switch is available at a low cost, Fig. 6A is a more preferred embodiment. Further, the conductors from the conductive loops to unit 3 should have a substantially small, fixed inductance to avoid adding artifacts to the inductance signals of interest. Preferably, these conductors are from small gauge coaxial cable.

Next, Fig. 6B illustrates an embodiment in which single demod and microp blocks are shared between three inductive plethysmographic loops, each loop having a dedicated oscillator. Preferably, the oscillators have digitized variable frequency output (*e.g.*, a variable frequency square wave), and switch SW may be a standard controllable digital switch. Here, local modules include the osc blocks. Fig. 6B is a preferred embodiment.

Finally, Fig. 6C illustrates a third embodiment in which only the microp block is shared and in which the local modules include both the osc and the demod blocks. This is the currently less preferred embodiment, but may be more advantageous where the osc and demod blocks can be implemented on a single mixed-type integrated circuit (IC).

The operation of the demod block is not described in more detail with reference to Fig. 7, in which portions of the embodiment of Fig. 6B is illustrated in more detail. In general, the demod block is a frequency counter which samples a digitized variable frequency input of from 200 to 400 kHz, and produces a digital frequency output accurate to at least 10 ppm (more preferably to 5 ppm, and even more preferably to 1 ppm). The output is preferably 24 bits or more. While any such frequency counter may be used in the present invention, Fig. 7 illustrates a particular such counter that can be economically implemented as a single programmed logic array IC.

Fig. 7 illustrates only two inductive plethysmographic loops, each with a dedicated osc block, OSC 1 and OSC 2, respectively. The digitized oscillator output is sampled by controllable switch SW and directed to the demod block components. Also input to the demod block is a 96 MHz clock signal. Other, preferably higher clock frequencies may be used, 96 MHz being merely a convenient frequency less than the about 120 MHz which the 80 ns logic in the current implementation is capable. The clock signal is divided to a 2 kHz clock by FREQ DIV block, which is first applied through an oscillator select block (OSC SEL) to control switch SW to sequentially sample the switched osc blocks for 0.5 ms (= 1/2kHz). The 2 kHz clock provides for a convenient sampling period, other sampling clock rates could be used. The microprocessor (MICRO-PROC) is also interrupted (IRQ lead) at the 2 kHz clock rate to accept the output digital data through a bus buffer and multiplexer (BUS MUX BUFFER), calculate the frequency from the accepted data, and optionally average two or more successive frequency measurements to determine frequencies in sample periods that are multiples of 0.5 ms, such as 2.5 ms. Finally, the 2 kHz clock is applied to counter, accumulator, and latch components (at the latch and reset, R, inputs) of the demod block to reset this circuitry for the next period of frequency measurement.

Generally, the circuit of Fig. 7 operates by counting the number of 96 MHz clock pulses that occur in the number of oscillator periods that occur in a particular 0.5 ms sampling interval (or sampling interval of other length). The components in oval 60 count

the oscillator periods in a sampling interval. The OSC CNT block is an 8 bit digital counter that counts the 200-400 kHz oscillator periods in the sampling interval, this count being latched into the OSC CNT LATCH and the counter reset every 0.5 ms. This eight bit count is supplied to the microprocessor through the BUS MUX BUFFER. Next, the components in oval 61 count the number of 96 MHz clock periods that occur in the counted number of oscillator periods. The TIMER CNT block is a 16 bit digital counter that counts clock periods. Since this count is latched into the TIMER CNT ACCUM block only on the occurrence of oscillator pulses applied to this block by lead 62, this accumulator block only contains counts of clock pulses within completed oscillator periods. At the end of a sampling period, the 16 bit count is latched into the TIMER LATCH, the counters are reset, and the 16 bit count is made available to the microprocessor through the BUS MUX BUFFER.

One of skill in the art will appreciate other equivalent circuit arrangements that are capable of obtaining these counts. In particular, not illustrated in Fig. 7 is hold circuitry, which prevents any activity for the first few (2-4) oscillator periods. This permits both accurate starting of the clock count as well as provides for circuit stabilization.

Finally, the MICRO-PROC divides the 8 bit oscillator period count by the 16 bit clock period count to obtain a 24 bit measured oscillator frequency. Note that it is oscillator period (the inverse of frequency) which varies directly with changes in cross sectional area as measured by the inductive loop.

Thus the demod block of Fig. 7 has a random error of one-half of a 96 MHz clock period, or 5.2 ns, during every 0.5 ms sampling interval. This is a less than 10 ppm-error that may be reduced by averaging; for example, averaging for 2.5 ms results in a less than approximately 2 ppm error. Thus the demod block of Fig. 7 achieves the accuracy required for inductive plethysmography. On the other hand, if the demod merely counted the number of oscillator cycles within a 0.5 ms sampling interval, then the random error would be one-half of an approximately 300 kHz period, or 1.67 μ s every 0.5 ms, of about 3300 ppm. Such inaccuracies would totally hide both respiratory and cardiac inductive plethysmographic signals, which at most have an amplitude to 1000 ppm.

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5.3. SYSTEMS AND METHODS

Fig. 10 illustrates overall methods of operation of a system according to the present invention incorporating a monitoring apparatus according to the present invention. Here, monitored individual 80 is illustrated as wearing a monitoring garment and holding (for data entry) a microprocessor unit (collectively, monitoring apparatus) according to preferred embodiments of the present invention.

The microprocessor unit may generate information and alarms directly to the monitored individual. Preferably, all data collected by the monitoring apparatus, including all raw data, is stored at a repository. Central repository 91, which is usually remote from the monitored individual, may store the data from many monitored individuals in server-type computer systems 86 equipped with database facilities. Monitored individuals may transmit data either by means of removable storage media 81a (such as a flash memory module) or by wireless transmission 81b, or by both means for different quantities and types of data.

Alternatively or additionally, data may be stored at local repository 92 after transmission by local wireless transmission 82. Removable media may also be used locally. In this case, the monitored individual's activities are usually restricted so that a substantial fraction of days are spent within a few hundred to a few thousand feet of the local repository. The local repository may be a PC-type computer with adequate storage, preferably hard disks of at least 10 GB capacity. Further the local repository may transmit stored data periodically to central repository 91 (if present).

Users of this system include monitored individual 80, who enters data on, *e.g.*, activities and symptoms into the microprocessor unit, and may receive medical reminders or alarms warning of conditions needing attention. Another type of user may be local health care professionals 85. These users may receive patient information (and transmit patient information and instructions) through local repository system 84. Local professionals may also receive information 93 from central health care professionals 90 by several means, such as telephone, e-mail, pager, and so forth. This information may provide patient status summaries or specific diagnostic and therapeutic guidance to the local professionals.

System users associated with the central repository include one or more central professionals 90, who advantageously access the system through local computers or terminals 89 in communication with server system 86. The central professionals oversee the medical status of all monitored individuals whose data is stored at that central repository. To assist these professionals the servers systems may be provided with display programs designed to conveniently survey the monitored population, and also with diagnostic or therapeutic programs which provide specific medical guidance perhaps by employing artificial intelligence techniques.

Also, monitoring users 88 are associated with central repository 91, which they access by local computers or terminals 87. These users oversee the technical operations of the monitoring apparatus of the monitored population, the operation of system computers and communications, and processing programs and resolve any problems. Monitoring users may also provide assistance to other system users in response to messages by telephone, e-

mail, or other means. Further, monitoring users may perform important quality control functions by overseeing the substantive function of the system of this invention. Quality control may include ensuring that the system correctly monitors physiological parameters, correctly displays and interprets monitored parameters to the various system users, and is in compliance with all regulatory requirements and guidance.

Finally, all system components incorporate security measures sufficient to meet mandated and preferable privacy requirements. These measures may include user authentication and data encryption as is known in the art.

10 **5.4. ADDITIONAL EMBODIMENTS**

Many additional embodiments will be apparent to one of skill in the art; come of which are described here.

Additional Inductive plethysmographic Sensors

15 Additional plethysmographic sensors may be incorporated in the monitoring apparatus of this invention. First, the data gathered by existing sensors can be augmented with additional sensors. For example, by adding one or more thoracic sensor bands superior to the already described cardiac band positioned at the level of the xiphoid process further information about cardiac function may be obtained.

Further, additional bands may provide new types of information. A sensor of a lower-abdominal cross-sectional area may be used to detect uterine contractions indicating the onset of labor in a pregnant female. Also, such a lower abdominal sensor may detect the present of intestinal gas and flatus.

25 An inductive plethysmographic sensor of a mid-abdominal cross-section, may monitor general intestinal activity. For example, absence of cross-sectional area variations may indicate a "silent abdomen," which is often a surgical emergency. Such monitoring may be advantageous in patients recovering from abdominal surgery.

Further, particular monitoring tasks may require higher accuracies or rates, or may permit reduced accuracies or rates. Appropriate accuracies and rates may be easily
30 determined by one of skill in the art in view of the monitoring task and the associated circuitry may be modified. For example, higher accuracies may be achieved by a demodulator circuit with higher clock frequencies or longer sampling intervals.

Generally, a garment may be "prescribed" for an individual according to his medical condition. Such a prescribed garment would have more or fewer sensors so that only
35 signals bearing on physiological parameters of interest are obtained and stored.

A Multi-Band Garment

A shirt-like garment may be constructed with a larger number of inductive plethysmographic sensor bands, for example, 10, or 20, or 30, or more bands, which are substantially uniformly distributed long the vertical axis of the torso. Signals from these
5 bands may be multiplexed into a smaller number of local electronic modules, which communicate with a microprocessor unit (or computer system) sufficiently capable to handle the increased data rate.

This larger number of signals may have several uses. First, they may be used for research in developing sensor bands for detecting additional physiologic parameters, or for
10 detecting current parameters with increased reliability for individual difficult to monitor because of activity or body habitus. Second, they can be used in selecting a cut and arrangement of a monitoring garment for particular individuals. An individual may wear a multi-band garment for a short time, and the associated processing unit may determine which bands are best at detecting desired physiological parameters. A final garment may
15 then be tailored with a cut, fit, and sensor band location best suited for that individual. This may replace garments fit to be adequate for a range of individuals, though ideal perhaps few or none. Third, a multi-band garment may be worn for regular monitoring, the few actual bands from which data is stored and communicated being selected in real time by the associated microprocessor unit as those with the best signals.

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Wireless Transmission Within A Single Monitoring Apparatus

With the advance of radio frequency (RF) circuitry and protocols, it may be advantageous to replace at least the primary signal cable by wireless transmission between the inductive plethysmographic sensors and the microprocessor unit. Optionally, all data
25 cables may be eliminated. This is advantageous to simplify use of the monitoring apparatus, with the monitoring garment, for a monitored individual.

Figs. 8A-B illustrate two principal (and exemplary) embodiments for such local wireless transmission. In these figures, the osc, demod, and microp blocks have the similar meanings to these blocks in Figs. 6A-C. The xmtr block is a RF transmitter; the rcvr block
30 is an RF receiver; and arrows 70 represent wireless transmission between the xmtr and the rcvr between the monitoring garment and its microprocessor unit. Preferably, the transmissions are of very low power in an un-licensed band, for example, the bands near 900 MHz or 2.4 Ghz. For actual implementation, it is preferable that the xmtr and rcvr blocks be implemented at least of single ICs, if not in a higher form of integration
35 embedded in other ICs.

In the embodiment of Fig. 8A, the digitized, 200-400 kHz output of the oscillators is transmitted from the modules associated with the plethysmographic sensors to the microprocessor unit. In order that variations in frequency be adequately demodulated, it is advantageous for a carrier-type timing signal be transmitted so that the xmtr and rcvr may be in phase lock. Circuitry similar to that used in portable phones may be employed.

In the embodiment of Fig. 8B, 8 and 16 bit digital words are transmitted from the sensors. Such digital transmission is advantageously by means of evolving standards and products for local digital transmission, such as the standard being developed by the Bluetooth consortium. In this embodiment, it may be advantageous to divide the microprocessor functions between a simple microprocessor, microp1, associated with each sensor, and a "central" microprocessor, microp2, in the microprocessor unit. Microp1 may losslessly compress the digital data to reduce wireless data rates and power requirement; microp2 then decompresses the received data. Compression may be as simple as subtraction of the oscillator 200-400kHz base frequency along with the transmission of runs of successive differences. Other compression techniques may be used.

Multiplexing of the RF transmission may be by frequency division, time division, or other multiplexing means known in the art.

Other division of the electronic function may be combined with local wireless transmission.

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In summary, while there have shown and described and pointed out fundamental novel features of the invention as applied to a preferred embodiment thereof, it will be understood that various omissions and substitutions and changes in the form and details of the devices illustrated, and in their operation, may be made by those skilled in the art without departing from the spirit of the invention. For example, it is expressly intended that all combinations of those elements and/or method steps which perform substantially the same function in substantially the same way to achieve the same results are within the scope of the invention. Moreover, it should be recognized that structures and/or elements and/or method steps shown and/or described in connection with any disclosed form or embodiment of the invention may be incorporated in any other disclosed or described or suggested form or embodiment as a general matter of design choice. It is the intention, therefore, to be limited only as indicated by the scope of the claims appended hereto.

The invention described and claimed herein is not to be limited in scope by the preferred embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended

to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

5 A number of references are cited herein, the entire disclosures of which are incorporated herein, in their entirety, by reference for all purposes. Further, none of these references, regardless of how characterized above, is admitted as prior to the invention of the subject matter claimed herein.

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WHAT IS CLAIMED IS:

1. A monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising:
 - 5 a monitoring garment comprising a shirt for the torso of the individual to be monitored,
one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional
10 area of the torso enclosed by the loop,
a cardiac cycle sensor for generating signals responsive to occurrence of cardiac ventricular contractions,
a signal cable for carrying signals from said sensors, and
a microprocessor unit comprising a microprocessor for receiving signals from said
15 signal cable and for recording digital data derived from all received signals in a removable computer-readable memory media.
 2. The apparatus of claim 1 wherein said cardiac cycle sensor comprises at least one electrocardiogram (ECG) electrode attached to said individual to be monitored.
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 3. The apparatus of claim 1 wherein said cardiac cycle sensor comprises at least one IP sensor closely fitting about the neck of said individual to be monitored, wherein signals the inductance of the IP sensor is responsive to cardiac ventricular contractions because the cross-sectional area of the neck is responsive to carotid artery pulsations generated by
25 cardiac ventricular contractions and the inductance of the IP sensor is responsive to the cross-sectional area of the neck.
 4. The apparatus of claim 1 wherein the computer-readable medium comprises a magnetic disk.
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 5. The apparatus of claim 1 wherein the computer-readable medium comprises a flash memory module.
 6. The apparatus of claim 5 wherein the flash memory module has a capacity of 64 MB or
35 more.

7. The apparatus of claim 1

wherein said monitoring garment further comprises a band for the neck of the individual to be monitored,

wherein said IP sensors comprise a neck inductive plethysmographic sensor
5 operatively arranged for generating signals responsive to jugular venous pulse, carotid arterial pulse, respiration-related intra-pleural pressure changes, contraction of neck muscles, and swallowing deflections, and

wherein the signal cable further comprises an attachment to the conductive loop of the neck IP sensor.

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8. The apparatus of claim 1 wherein said IP sensors comprise at least one abdominal IP sensor including one or more conductive loops and at least one rib cage IP sensor including one or more conductive loops operatively arranged for measuring breathing patterns of the patient.

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9. The apparatus of claim 1 wherein said IP sensors comprise at least one thoracic IP sensor including one or more conductive loops operatively arranged for measuring ventricular stroke volume.

20 10. The apparatus of claim 1 wherein said IP sensors comprise at least one lower abdominal IP sensor operatively arranged for measuring intra-lower-abdominal contractions and dilations.

11. The apparatus of claim 1 wherein said IP sensors comprise one or two hemithoracic IP
25 sensors operatively arranged for measuring breathing and paradoxical motion between two hemithoraces of the patient.

12. The apparatus of claim 1 further comprising one or more further sensors attached to the signal cable and selected from a group comprising a body position sensor for indicating a
30 posture of the individual, a pulse oximeter for indicating arterial oxygenation saturation, and a throat microphone for indicating talking and snoring.

13. The apparatus of claim 12 further comprising at least two body position sensors, a first body position sensor mounted on said garment and a second body position sensor mounted
35 elsewhere on the individual.

14. The apparatus of claim 1 wherein said IP inductive plethysmographic sensors are attached to said garment as an integral part of said garment via an attachment consisting of one of sewing, embroidering, embedding, weaving and printing said inductive plethysmographic sensor into said garment.
- 5
15. The apparatus of claim 1 wherein said microprocessor unit further comprises an audio device for generating audio indications to the individual being monitored.
16. The apparatus of claim 1 wherein said microprocessor unit further comprises a display
10 unit for displaying viewable messages to the individual being monitored.
17. The apparatus of claim 1 wherein said microprocessor unit further comprises an input unit for the individual being monitored to input information or commands to said microprocessor unit.
- 15
18. The apparatus of claim 1 wherein said microprocessor unit further comprises a memory accessible to the microprocessor, and wherein the memory comprises encoded software instructions for causing the microprocessor to read input data and to write output data derived from the input data in the removable computer-readable memory media.
- 20
19. The apparatus of claim 18 wherein the memory further comprises encoded software instructions for causing the microprocessor to determine significant physiological events in the individual being monitored and to indicate audibly determined significant events to the individual.
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20. The apparatus of claim 19 wherein the microprocessor unit comprises components for wirelessly transmitting determined events.
21. The apparatus of claim 18 wherein the memory further comprises encoded software
30 instructions for causing the microprocessor to determine significant temporal physiological trends in the individual being monitored and to indicate audibly determined significant trends to the individual.
22. The apparatus of claim 21 wherein the microprocessor unit comprises components for
35 wirelessly transmitting determined significant trends.

23. The apparatus of claim 18 wherein the memory further comprises encoded software instructions for causing the microprocessor to compress data before writing to the removable computer-readable memory media.

5 24. The apparatus of claim 1 wherein the microprocessor unit further comprises circuitry for deriving digital data from non-digital data received from the signal cable.

25. The apparatus of claim 1 wherein said monitoring apparatus further comprises circuitry for generating a variable-frequency signal from each IP sensor, the generated frequency
10 being responsive to the inductance of the conductive loop of the IP sensor, and wherein the microprocessor unit further comprises circuitry for deriving digital data from the generated variable-frequency signals, the digital data comprising encoding of the variable frequency of the signals with errors of 100 ppm or less.

15 26. A monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising:

a monitoring garment comprising a shirt for the torso of the individual to be monitored,

one or more inductive plethysmographic (IP) sensors, each IP sensor comprising
20 (i) a longitudinal band of elastic material attached to said garment for closely encircling the torso,

(ii) an inductance sensor including at least one flexible conductive loop attached to the longitudinal band, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop, and

25 (iii) a tightening device for adjusting circumferential tightness of the IP sensor to substantially prevent longitudinal movement of the IP sensor along the torso, and
a microprocessor unit comprising a microprocessor for receiving signals from said IP sensors and for recording digital data derived from all received signals in a removable computer-readable memory media.

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27. The apparatus of claim 26 wherein longitudinal motion of each IP sensor is substantially prevented when the physiological parameters indicated by the inductance of the conductive loop of the sensor do not measurably change.

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28. The apparatus of claim 26 wherein the monitoring garment comprises excess fabric arranged to permit longitudinal stretching of the torso without applying force to the IP sensors sufficient to cause substantial longitudinal motion.
- 5 29. The apparatus of claim 28 wherein longitudinal motion of each IP sensor is substantial if physiological parameters indicated by the inductance of the conductive loop of the sensor change as the monitoring garment is worn by the individual.
30. The apparatus of claim 26 wherein the monitoring garment comprises fabric with
10 sufficient longitudinal elasticity to permit longitudinal stretching of the torso without applying force to the IP sensors sufficient to cause substantial longitudinal motion.
31. The apparatus of claim 26 wherein the tightening device comprises a cinch band and a gripping device for releasably gripping excess cinch band under tension.
- 15 32. The apparatus of claim 26 wherein the tightening device comprises a drawstring.
33. The apparatus of claim 26 further comprising a cardiac timing sensor for generating signals responsive to cardiac ventricular contractions, and wherein said microprocessor unit
20 further records digital data derived from signals received from said cardiac timing sensor.
34. The apparatus of claim 26 further comprising a signal cable for carrying signals from said sensors to said microprocessor unit.
- 25 35. A monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising:
a monitoring garment comprising a shirt for the torso of the individual to be monitored and a longitudinal fastener for opening and closing the shirt,
one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an
30 inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop,
a cardiac timing sensor for generating signals responsive to occurrence of cardiac ventricular contractions,
35 a signal cable for carrying signals from said sensors comprising at least one module, wherein the module is coupled to and electrically completes the conductive loops of the IP

sensors, wherein termini of the conductive loops may be uncoupled from module, and wherein the module comprises circuitry for generating signals responsive to the IP sensors, and

5 a microprocessor unit comprising a microprocessor for receiving signals from said signal cable and for recording digital data derived from all received signals in a removable computer-readable memory media.

36. The apparatus of claim 35 wherein at least one IP sensor further comprises a tightening device for adjusting circumferential tightness of the IP sensor to substantially prevent
10 longitudinal movement of the IP sensor along the torso, and wherein the tightening device can be arranged not to impede unfastening of the shirt.

37. The apparatus of claim 35 wherein the conductive loops of the IP sensors and the module further comprise mating connectors so that the conductive loops may be connected
15 and disconnected from the module.

38. The apparatus of claim 35 wherein the signals generated by the module in response to each IP sensor comprise digital data encoding the frequency of an oscillator responsive to the inductance of the conductive loop of the IP sensor, the frequency being encoded with
20 errors of 100 ppm or less.

39. The apparatus of claim 38 wherein errors of frequency encoding are 10 ppm or less.

40. The apparatus of claim 35 wherein the signals generated by the module in response to
25 each IP sensor comprise signals of variable frequency, the frequency being responsive to the inductance of the conductive loop of the IP sensor.

41. The apparatus of claim 40 wherein the microprocessor unit further comprises circuitry for deriving digital data from the variable-frequency signals generated from each IP sensor,
30 the digital data comprising encoding of the variable frequency of the signals with errors of 100 ppm or less.

42. The apparatus of claim 41 wherein the microprocessor unit further comprises multiplex circuitry for permitting single deriving circuitry to derive digital data from a plurality of
35 variable-frequency signals.

43. A monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising:

- 5 a monitoring garment comprising a shirt for the torso of the individual to be monitored,
- one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the cross-sectional area of the torso enclosed by the loop,
- 10 a cardiac timing sensor for generating signals responsive to occurrence of cardiac ventricular contractions,
- a signal cable for carrying signals directly from the conductive loops of said IP sensors and for carrying signals from said sensor,
- electronic circuitry comprising (i) a multiplexing switch for connecting the
- 15 conductive loop of any one of the IP sensors to an oscillator, the oscillator having an oscillation frequency responsive to the inductance of the conductive loop connected by the multiplexing switch, and (ii) a demodulator operatively coupled to the oscillator and outputting digital data responsive to the oscillation frequency, and
- a microprocessor unit comprising a microprocessor for receiving signals from said
- 20 signal cable and for receiving digital data from said electronic circuitry and for recording digital data from received inputs in a removable computer-readable memory media.

44. The apparatus of claim 43 wherein the digital data responsive to the oscillation frequency has errors of 100 ppm or less.

25 45. The apparatus of claim 43 wherein the digital data responsive to the oscillation frequency has errors of 10 ppm or less.

46. The apparatus of claim 43 wherein said electronic circuitry is housed in said

30 microprocessor unit.

47. The apparatus of claim 43 wherein the resistance of the data signal cables and the multiplexing switch from the conductive loop of any IP sensor to the oscillator is less than 1 Ω .

35

48. The apparatus of claim 43 wherein the multiplexing switch is controlled so that oscillator is periodically connected to the conductive loop of each IP sensor for the duration of a sampling period.

5 49. The apparatus of claim 48 wherein the sampling period is 1 msec or less.

50. The apparatus of claim 48 wherein the digital data output by the demodulator comprises digital data encoding a count of a number cycles of the oscillator occurring within a sampling period and digital data encoding a count of a number of periods of a clock
10 occurring within the counted oscillator cycles.

51. The apparatus of claim 50 wherein said microprocessor unit further comprises a memory accessible to the microprocessor, and wherein the memory comprises encoded software instructions for causing the microprocessor to determine the actual oscillator
15 frequency by dividing the count of the number of oscillator cycles by the count of the number of clock periods.

52. The apparatus of claim 51 wherein the memory further comprises software instructions for causing the microprocessor to determine an more accurate frequency by combining the
20 counts of a plurality of sampling periods.

53. A monitoring apparatus for non-invasively monitoring physiological parameters of an individual comprising:
a monitoring garment comprising a shirt for the torso of the individual to be
25 monitored,
a plurality of sensors, said sensors comprising
(i) one or more inductive plethysmographic (IP) sensors, each IP sensor comprising an inductance sensor including at least one flexible conductive loop arranged to closely encircle the torso, wherein the inductance of the conductive loop is responsive to the
30 cross-sectional area of the torso enclosed by the loop,
wherein at least one sensor comprises a transmitter for wirelessly transmitting signals generated by the sensor within the vicinity of said physiological monitoring apparatus,
a microprocessor unit comprising (i) a receiver for receiving signals wirelessly
35 transmitted from said sensors, and (ii) a microprocessor for accepting the received signals

and for recording digital data derived from the received signals in a removable computer-readable memory media.

54. The apparatus of claim 53 wherein at least one sensor generates output signals in a digital form, and wherein the transmitter transmits the generated digital signals.

55. The apparatus of claim 54 wherein the transmitter and the receiver conform to the Bluetooth standard.

56. The apparatus of claim 53 wherein at least one sensor generates variable-frequency analog output signals, and wherein the transmitter output is modulated by generated variable-frequency analog signal.

57. The apparatus of claim 53 wherein all sensors comprise a transmitter for wirelessly transmitting signals generated by the sensor within the vicinity of said physiological monitoring apparatus.

58. The apparatus of claim 53 further comprising a signal cable, wherein the output of at least one sensor is carried to said microprocessor unit by a signal cable, and wherein said microprocessor records digital data derived from signals carried by said signal cable.

59. The apparatus of claim 53 wherein said sensors further comprise a cardiac timing sensor for generating signals responsive to occurrence of cardiac ventricular contractions.

60. A system for the non-invasive physiological monitoring of physiological parameters of at least one individual comprising:

at least one physiological monitoring apparatus comprising a monitoring garment worn on the torso of an individual being monitored, wherein the monitoring apparatus stores in a digital form in a removable computer-readable memory media data, wherein the data is by sensors comprising generated from (i) one or more inductive plethysmographic (IP) sensors flexibly attached to the monitoring garment, and (ii) a cardiac timing sensor for generating signals responsive to cardiac ventricular contractions, and

a data repository for reading data from the removable computer-readable memory media that has been recorded by said physiological monitoring apparatus and for storing read data in a data archive, said data repository being remotely located from said physiological monitoring apparatus.

61. The system of claim 60 wherein said physiological monitoring apparatus further transmits data wirelessly, and wherein said data repository further receives data wirelessly that has been transmitted by said physiological monitoring apparatus, and then stores the received data.

5

62. The system of claim 61 wherein said physiological monitoring apparatus further comprises a microprocessor for processing the generated data for determining physiological events and alarms, and wherein the data wirelessly transmitted comprises the determined physiological events and alarms.

10

63. The system of claim 61 further comprising a local data repository co-located with said physiological monitoring apparatus, wherein the local data repository receives data wirelessly transmitted by said physiological monitoring apparatus and stores received data in a local data archive, and wherein the local data repository comprises display terminals for making stored data available to local health care professionals.

15

64. The system of claim 60 wherein said data repository further comprises display terminals for making stored data available to health care professionals and to users monitoring the operation of said system.

20

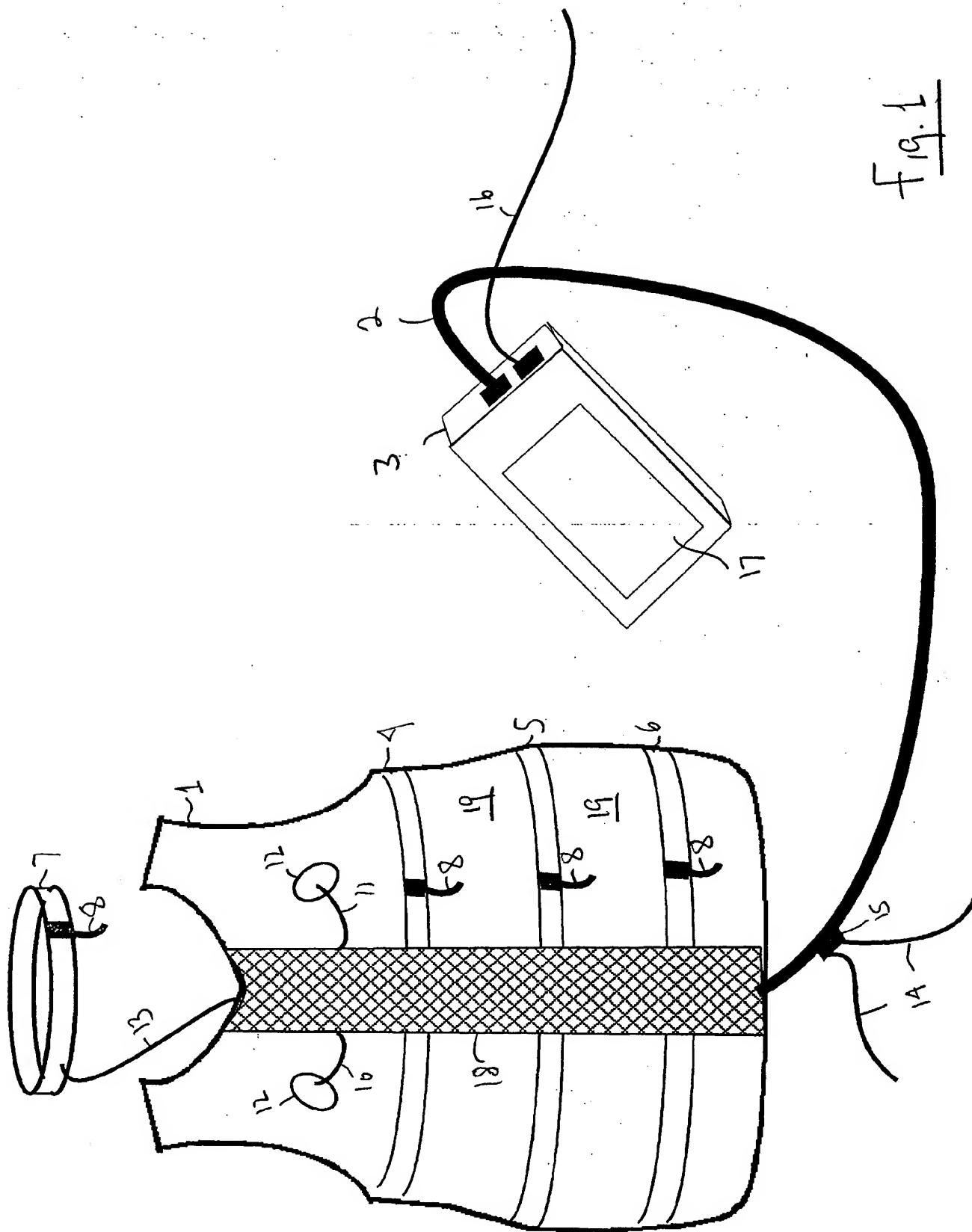
65. The system of claim 60 further comprising a plurality of physiological monitoring apparatus, each apparatus for monitoring a different individual, and wherein said data repository reads data from removable computer-readable memory media recorded by said plurality of physiological monitoring apparatus.

25

67. A computer readable medium comprising data recorded in digital form, wherein the recorded digital data comprises data responsive with errors of 100 ppm or less to the frequency of an oscillator connected to at least one conductive loop of at least one inductive plethysmographic sensor.

30

35



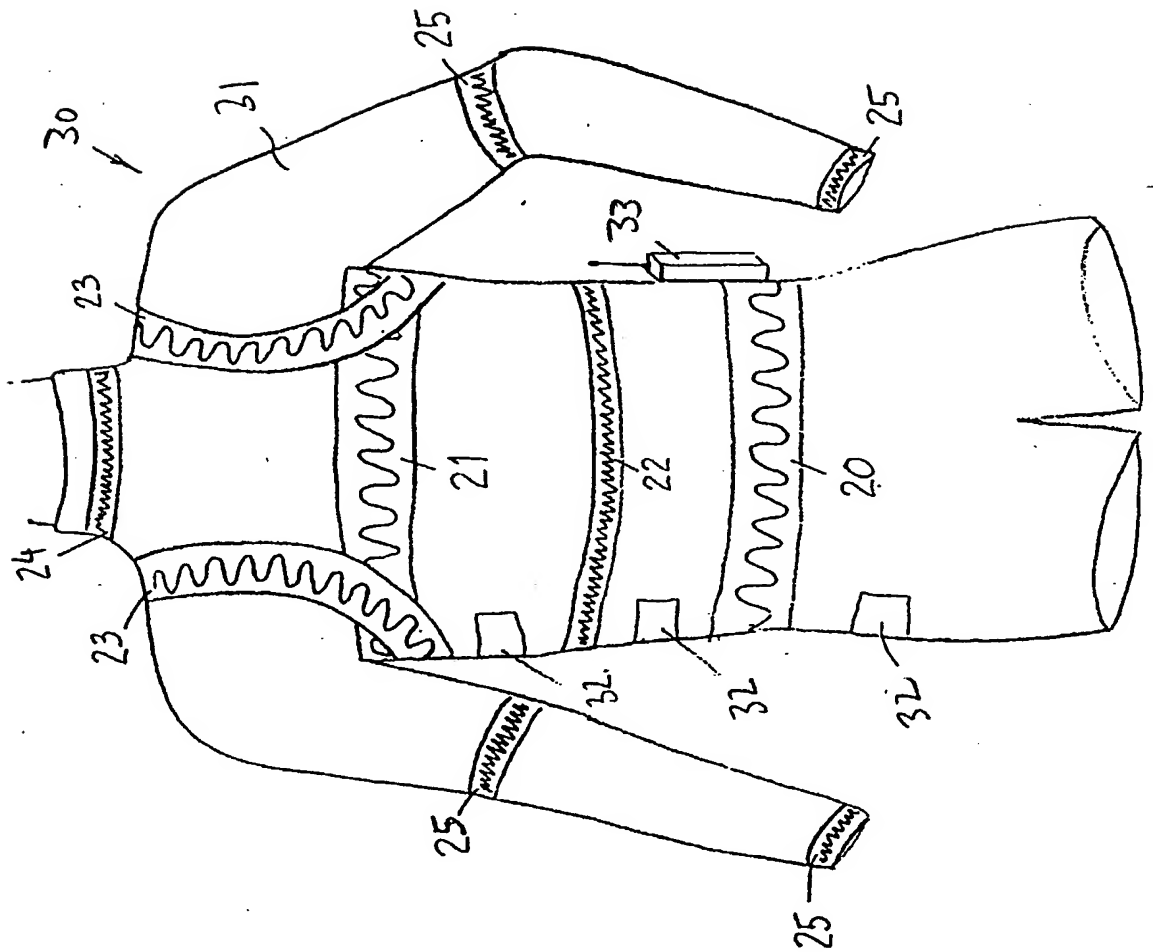


Fig. 2

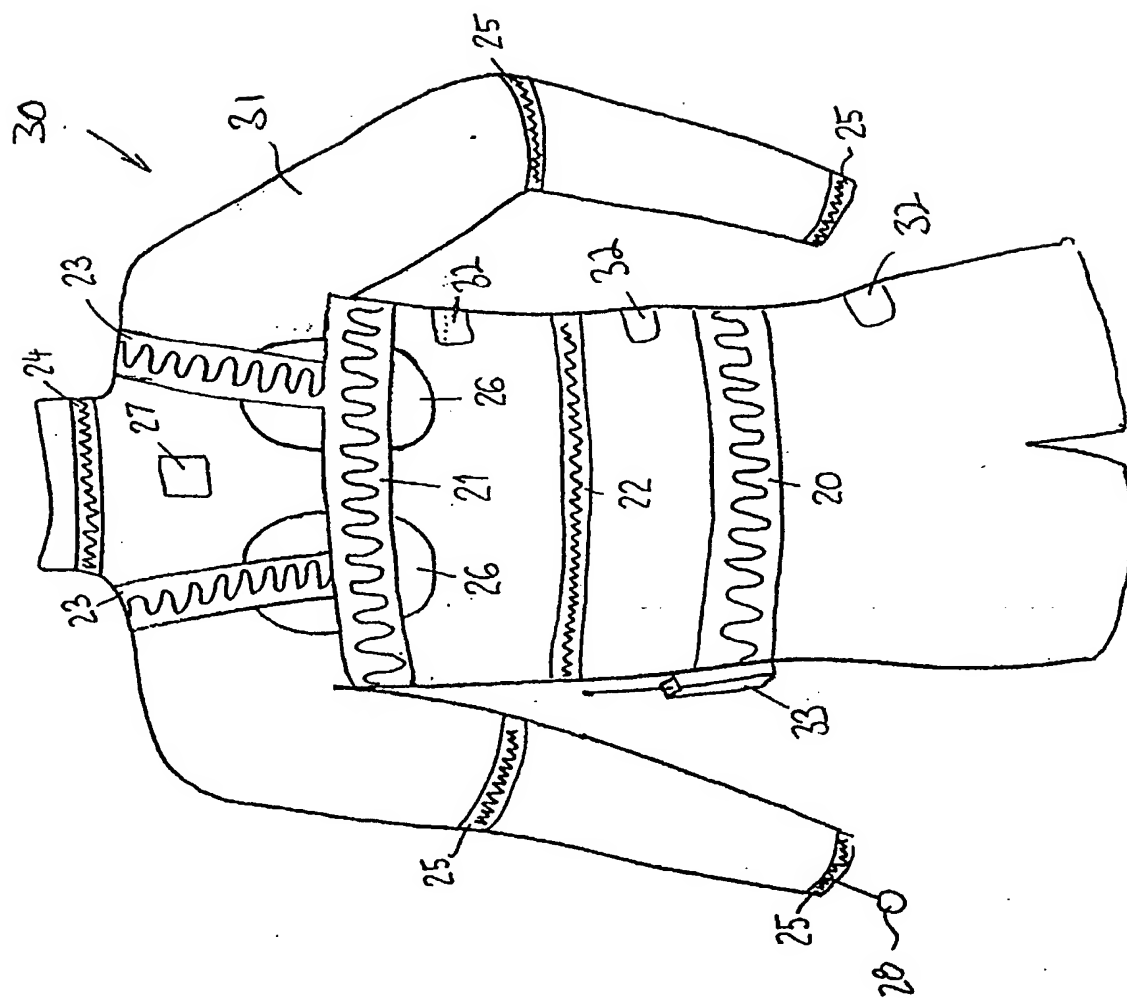


Fig. 3

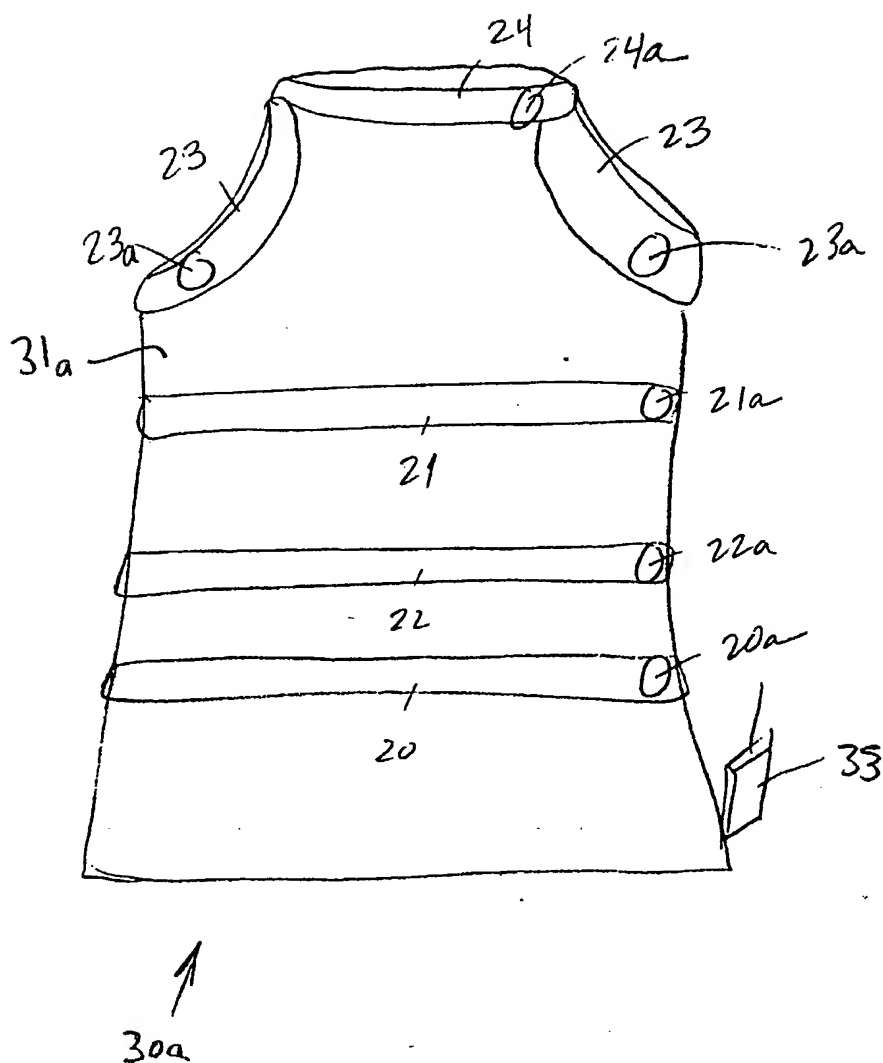
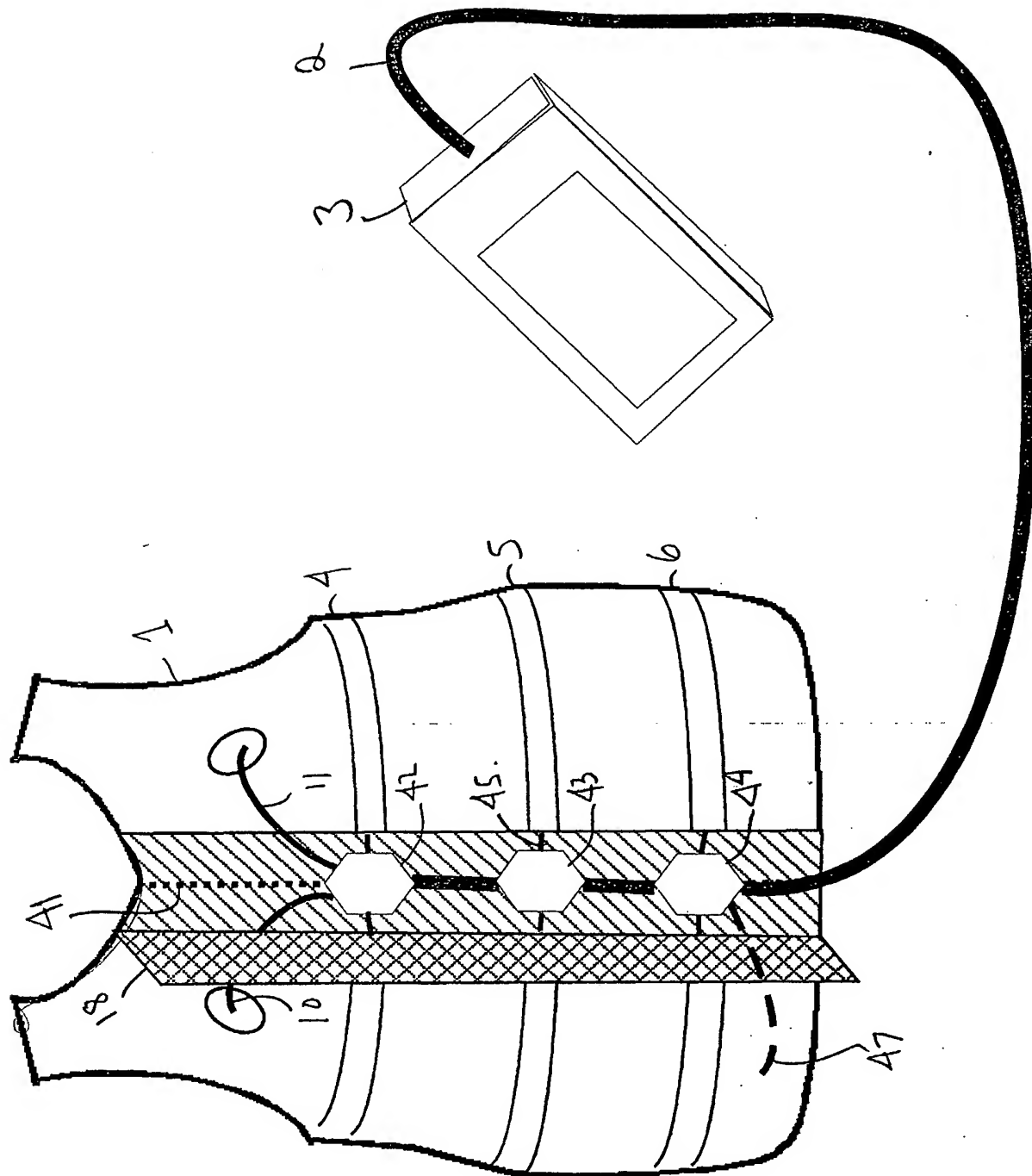


fig. 4

Fig. 5



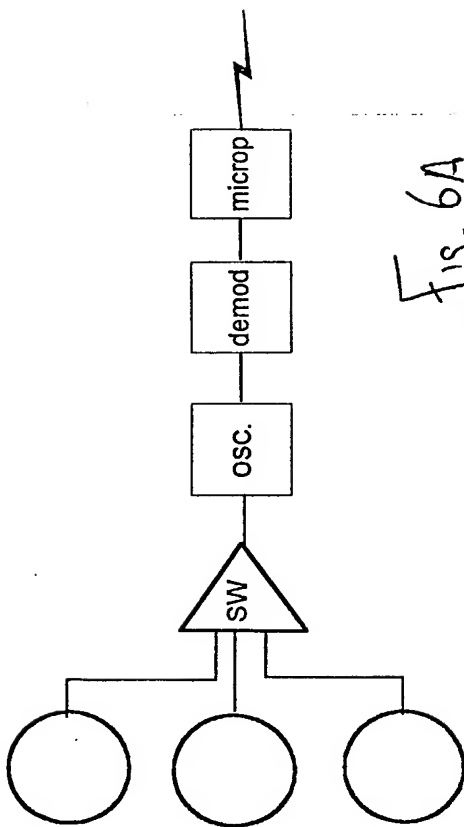


Fig. 6A

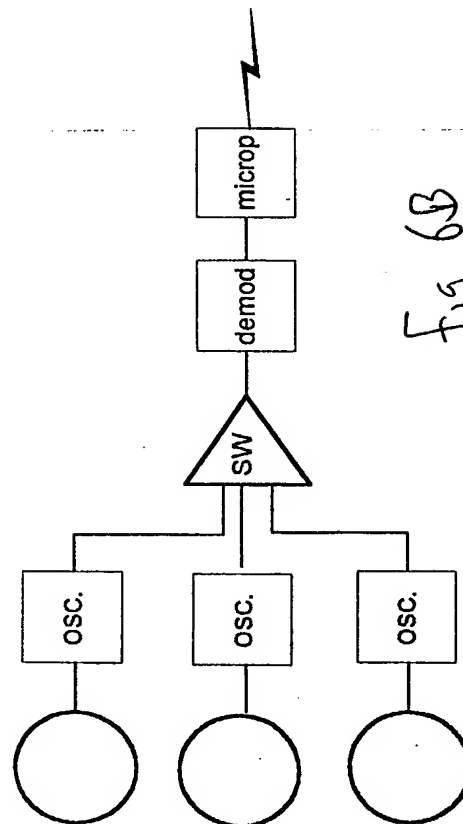


Fig. 6B

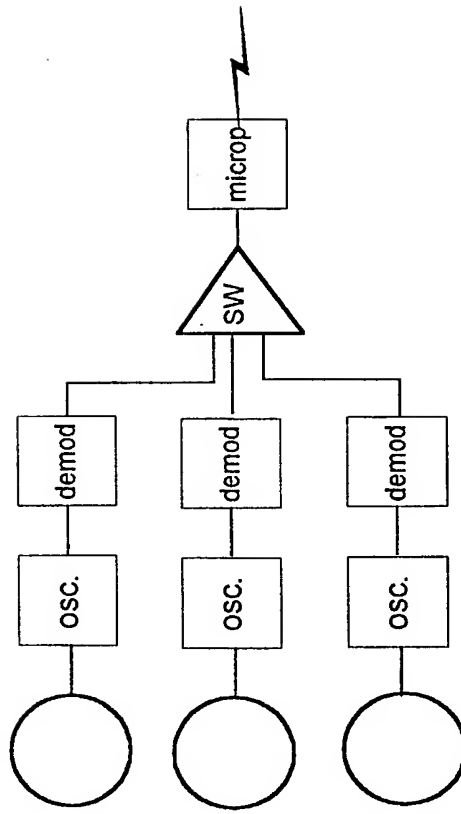


Fig. 6C

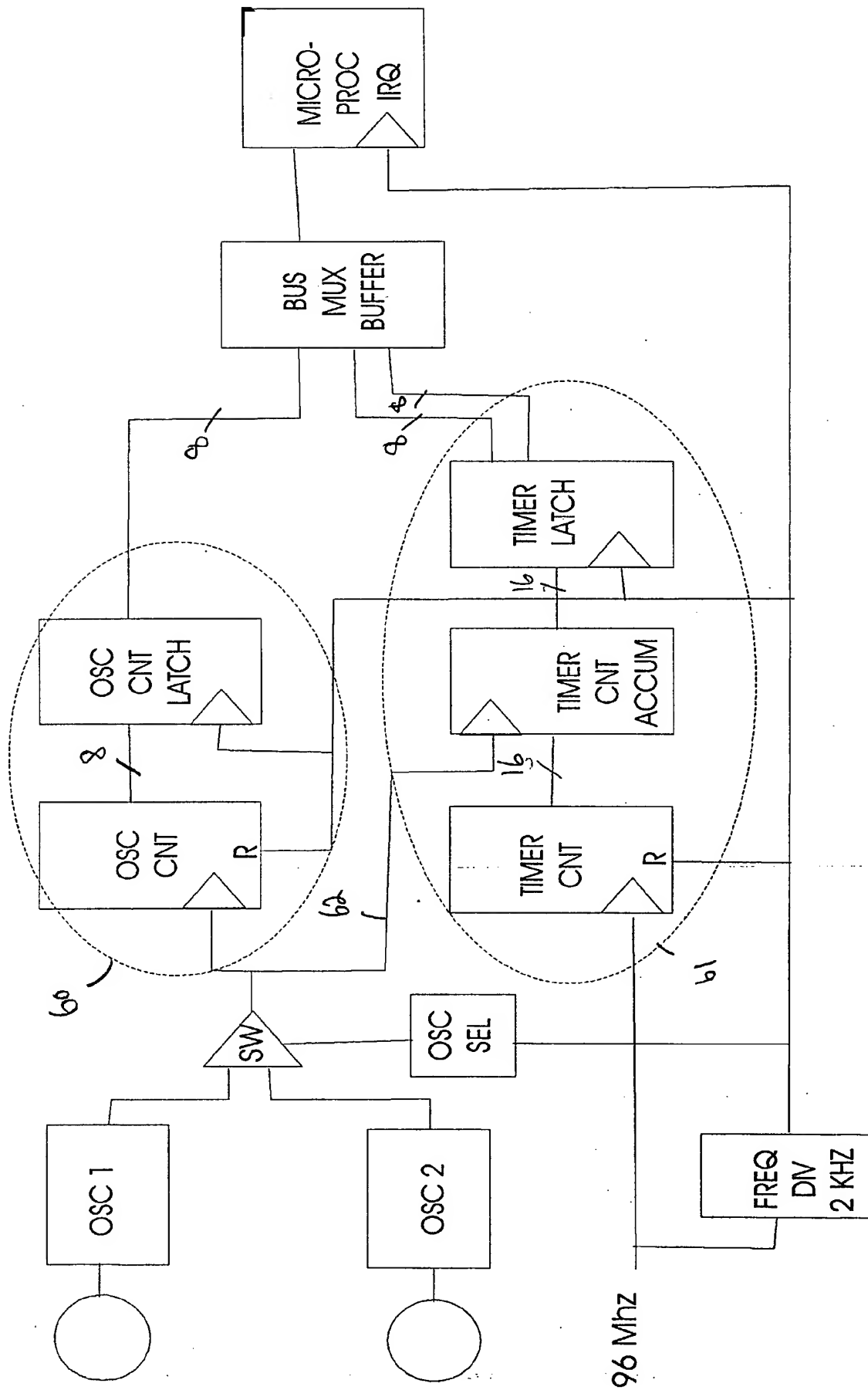
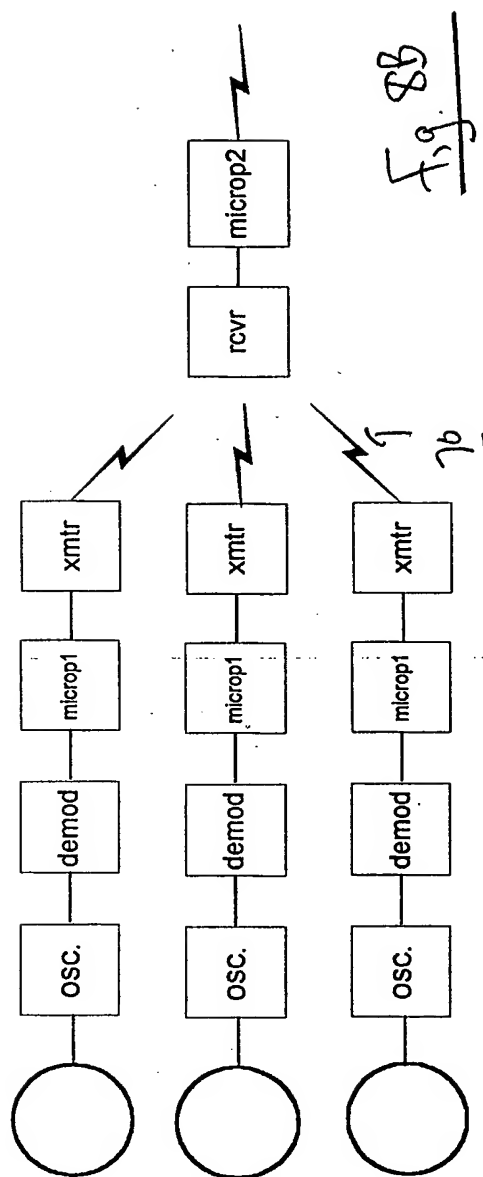
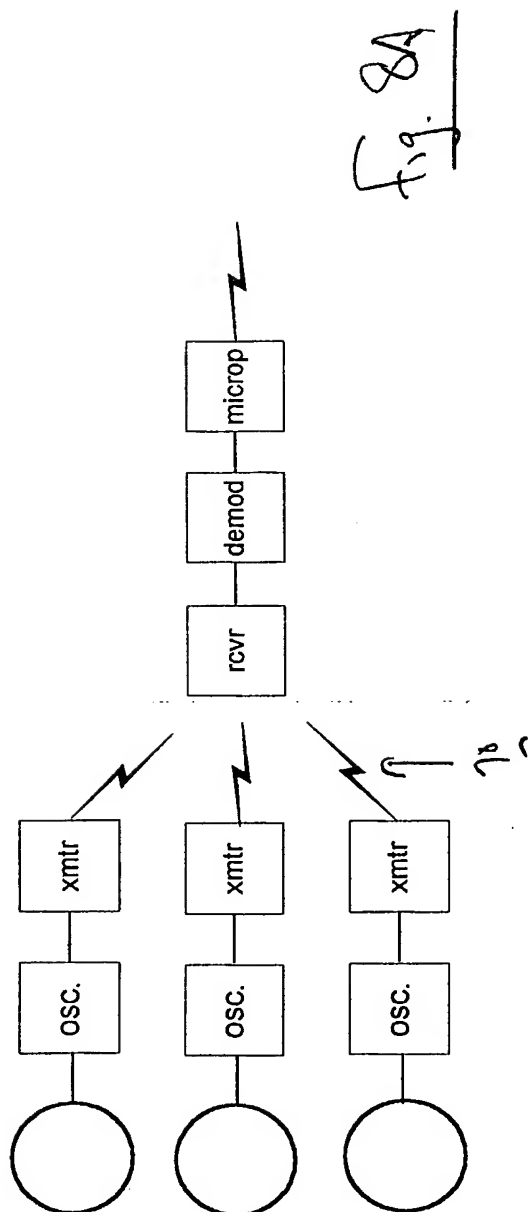


Fig. 7



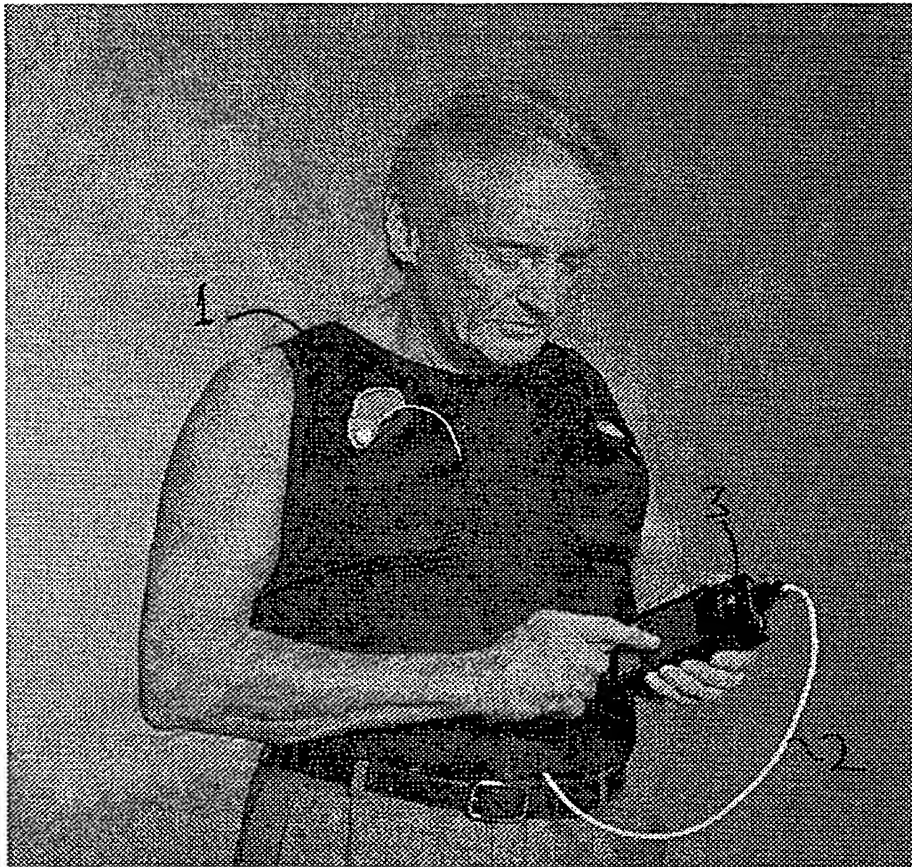


Fig. 9

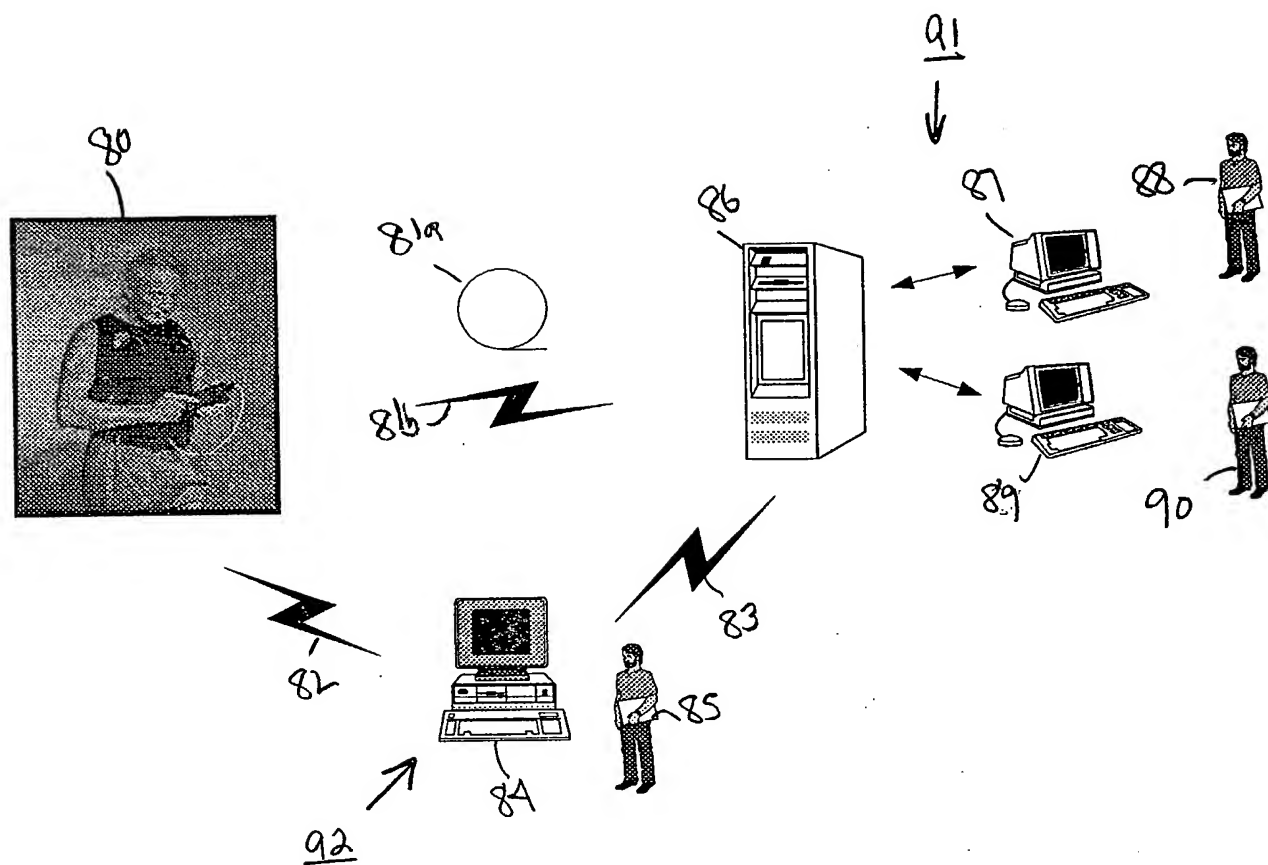


Fig. 10.

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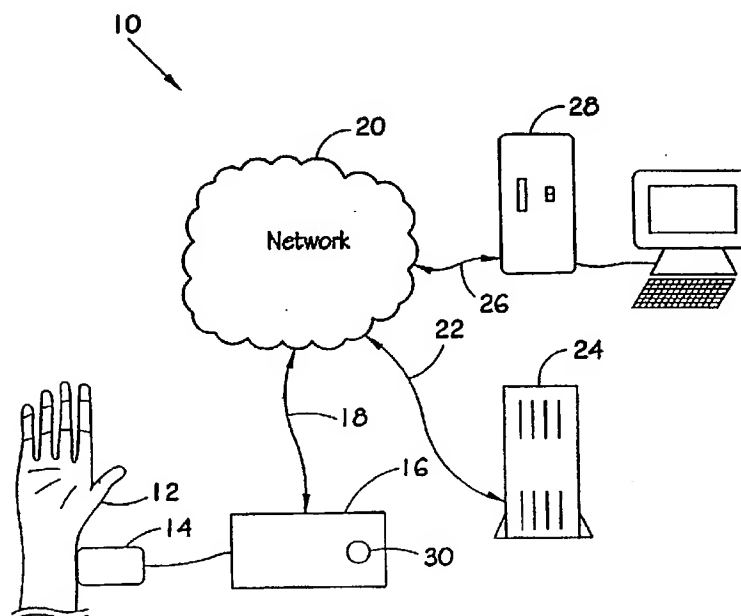
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(54) Title: GLUCOSE MONITORING INSTRUMENT HAVING NETWORK CONNECTIVITY



(57) Abstract: A glucose monitoring instrument having network-based communication features which provide a link between patient and practitioner. The glucose monitoring instrument comprises circuitry for communicating data with one or more destination sites on the network which are configured to transmit and receive information to and from the instrument. Instrument measurements are transmitted over the link in addition to information and guidance, to provide increased accuracy, improved program compliance, and patient guidance from a supervisory authority or medical practitioner. In addition, a set of calibration features encourage calibration compliance.

GLUCOSE MONITORING INSTRUMENT HAVING NETWORK CONNECTIVITY

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention pertains generally to medical equipment for measuring blood glucose levels, and more particularly to a blood glucose measuring instrument having internet-based communication features.

2. Description of the Background Art

A patient, having been taught how to use an existing portable glucose monitor is generally required thereafter to independently conduct and record their own measurements. Furthermore, the patient typically is required to both record and assess the measurements without benefit from their practitioner or a supervising authority. Numerous errors can arise from these unsupervised procedures that may result in serious health risks for patients which knowingly, or inadvertently, are not in compliance with medical directives.

Typically, patients using a glucose monitor are given a schedule of measurements to be complied with and a notebook in which to record the measurements. Patients often forget, or in some instances forego, conducting and correctly recording their glucose levels as measured by the instrument. If a patient skips a measurement they may even elect to write down a "likely" number in the notebook as if such a measurement had been taken. Patient interaction with such a manual glucose monitoring instrument therefore provides no assurance of correct measurement and recordation. Furthermore, patients in a myriad of situations may require additional information and assistance with regard to the use and maintenance of their glucose measurement instrument.

In addition, to assure glucose measurement accuracy, a measuring instrument may require periodic calibration and assuring calibration compliance on instruments in the field is burdensome.

Therefore, a need exists for a glucose monitoring system that provides a link between the patient and the practitioner to encourage compliance and facilitate equipment calibration. The present invention satisfies those needs, as well as others, and overcomes deficiencies in current monitoring systems and procedures.

BRIEF SUMMARY OF THE INVENTION

The present invention is a glucose monitoring device with remote communications capabilities. According to an aspect of the invention, a data link is provided between the equipment and a centralized station, or server. The centralized station can monitor important information, such as: equipment calibration, the diligence of a patient taking and recording measurements according to a schedule, and the actual measurements taken by the patient. The centralized station is preferably capable of forwarding information to the patient's physician for evaluation. In addition, the centralized station can have optional capability of locking out the patient if the patient has not paid his or her bills. According to another aspect of the invention, the information is communicated from the glucose monitor directly to the physician. As can be seen, therefore, the invention links the monitoring activities performed by the patient and the assessment of those activities by the physician while reducing the chance of human error introduced into the long-term monitoring and treatment process.

By way of example, and not of limitation, a non-invasive subsurface spectrophotometer instrument equipped with a communications link according to the invention takes the glucose measurements and communicates them over a network, such as the internet. The spectrophotometer instrument comprises data communication circuitry, such as dial-up circuitry, and additional session control protocols which integrate a number of the functions within the instrument for communication over a network connection. A destination site, or sites, on the network are configured to receive information from the instrument and to transmit information and services.

An object of the invention is to provide a link from patient to practitioner over which timely communication of pertinent glucose monitoring information can travel.

Another object of the invention is to provide for remote monitoring of patient compliance.

Another object of the invention is to provide for remote equipment monitoring and calibration by a central station.

Another object of the invention is to provide for periodic transmission of measurement results.

Another object of the invention is to provide network communication over a standardized communications interface.

Another object of the invention is to provide a communications structure which can support data encryption.

Another object of the invention is to aid compliance by alerting the patient, by sound or visual cues, when the time arrives to conduct a measurement.

Another object of the invention is to reduce human error in conducting and recording measurements.

Another object of the invention is to eliminate secretive non-compliance, wherein a patient enters fictional measurement data into the measurement log.

Another object of the invention is to provide the capability of generating programmed practitioner warnings when measurements fall outside the bounds of a selected range.

Another object of the invention is to provide a panic button which allows a concerned patient to alert their practitioner.

Another object of the invention is to engender practical practitioner guidance to patients.

Another object of the invention is to allow the instrument manufacturer to track compliance and calibration of the glucose monitoring instrument.

Another object of the invention is to provide an accurate database that may be used by insurance and pharmaceutical companies.

Further objects and advantages of the invention will be brought out in the following portions of the specification, wherein the detailed description is for the purpose of fully disclosing preferred embodiments of the invention without placing limitations thereon.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be more fully understood by reference to the following drawings which are provided for illustrative purposes only:

FIGURE 1 is a functional block diagram showing a glucose monitoring system with network connectivity according to the present invention.

FIGURE 2 is a block diagram of the electronic circuits within the glucose monitoring system of FIG. 1.

FIGURE 3 is a flowchart exemplifying calibration lockout according to one aspect of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Referring more specifically to the drawings for illustrative purposes, the present invention is embodied in the system generally shown in FIG. 1 through FIG. 3. It will be appreciated that the system may vary as to configuration and as to details of the parts, and that the method may vary as to the specific steps and sequence, without departing from the basic concepts as disclosed herein.

Referring to FIG. 1, a glucose monitoring system 10 is shown connected to remote stations over a network. The hand of a patient 12 is shown positioned to allow a measurement to be performed by glucose detector element 14 which is capable of taking non-invasive measurements of bodily glucose levels. Preferably, measurement element 14 comprises a thermal gradient detector such as a spectrophotometer. A signal processing system 16 of the instrument collects the measurements from detector 14 and processes the data into a set of results. It will be appreciated that the instrument preferably includes input and output devices, such as a display and a set of control inputs (not shown) for communicating information directly to and from the patient. The combination of detector 14 and processor 16 form a complete non-invasive glucose measuring device that typically would be used in the home of a patient. Examples of glucose monitoring equipment without internet connectivity are described in U.S. Patent Numbers 5,900,632 to Sterling et al. and 5,615,672 to Braig et al., which are both incorporated herein by reference. In accordance with the present invention, however, the signal processing system is equipped with a network interface along with one or more processing elements for processing the measurement signals and for control of network communications.

Data is communicated over the network as determined by the configuration of the system and the state and condition of the measurement being performed. Measurement data may accordingly be communicated to a remote station at the time the measurement is performed, or it may be retained within the instrument and sent to the remote station according to a schedule or other selection criterion. The instrument is capable of comparing each measurement with a set of limits and providing alerts to a supervisory authority regarding excursions therefrom. In FIG. 1 the measurement data is shown being routed through a connection 18 to the internet 20, whose destination is routed through connection 22 to a centralized monitoring computer 24, or a server. The centralized computer is preferably capable of checking the data for emergency conditions and logging the data for later use. In addition, the centralized computer may monitor equipment status for proper operation and calibration. It will be appreciated that multiple servers, or centralized stations, can be provided for communicating

with the blood glucose instruments. Furthermore, the centralized computer may transfer or simultaneously route the data via connection 26 to a computer 28 in the office of a medical practitioner over the internet. Alternatively, the data may be directly sent over the internet to an associated medical practitioner 28 from processor 16. It will be appreciated that the foregoing data routing is provided as an example, and not as a limitation, of the data routing utilized to provide the internet services as described according to the invention. Preferably a panic button 30 provides the patient with a mechanism for alerting a medical practitioner should an important concern arise. In addition, sound and/or visual output is preferably provided by the instrument for signaling the patient when the time arrives to perform a measurement, or of a directive from a supervisory authority as received over the internet.

The present invention, as described herein, provides either a direct or indirect link from the patient to the practitioner. The practitioner is thereby accorded an ability to monitor the status of the patient and may elect to be alerted should deviations in the measurement values or timeliness arise. The system may be configured to transmit measurement data at predetermined intervals, or at the time each measurement is performed. The measurements can be transmitted using various network protocols which include standard internet protocols, encrypted protocols, or email protocols.

In the preferred embodiment of the invention, processor 16 is additionally capable of providing visual or audible cues to the patient when the time arrives to conduct a measurement. These alerts may be augmented by requests, over the internet link of the instrument, from the practitioner. Errors introduced within measurements and recordation within a manual system can thereby be eliminated with the electronically logged measurements. It will be appreciated that the system provides enhanced utility and measurement credibility over the use of an instrument that requires manual logging of the measurements and no practitioner interaction thereof.

Secretive non-compliance may also be eliminated as the patient is not conferred the responsibility of manually logging measurements. In using the instrument according to the present invention, the measurements collected within the instrument by the patient are capable of being transmitted to the practitioner, or centralized station, such that if a patient is not being diligent in conducting measurements, the practitioner may immediately contact the patient to reinforce the need for compliance. In addition, the information provided over the network can be used to warn the practitioner when measurement readings appear abnormal, so that the practitioner may then investigate the situation and verify the status of the patient.

It will be appreciated that the invention has particular utility for patients preferring to receive direct guidance from a practitioner. The information that flows between the patient and the practitioner increases the ability of the practitioner to provide knowledgeable patient guidance.

FIG. 2 illustrates the functional blocks of an embodiment of circuitry 32 for implementing the signal processing hardware 16 shown in FIG. 1. A network connection 34 connects to a network processing circuit, exemplified by an Internet Protocol (IP) circuit or processor 36. Numerous circuits are available for providing internet connectivity, such as the SX-Stack[®] chip from Scenix Semiconductor, and the iChip[®] from Connect One Electronics. These integrated circuit chips and other available chips provide interface layers for supporting a Transmission Control Protocol/Internet Protocol (TCP/IP). The internet protocol chip 36 has an interface 38 with

a control processor section 40, which preferably comprises microcontroller or like. Control processor section 40 in turn has access to conventional memory 42. To provide security and fault tolerance of the instrument it is preferable for the control processor, or the internet protocol circuit, to encrypt and provide verification strings or tokens within the data being sent across the network, and accordingly to decrypt information being received and
5 verify the received strings or tokens. The control processor 40 has an interface 44 with the instrumentation circuits 46, which is in turn configured with an interface 48 to the glucose detection element 14 shown in FIG. 1.

The network link provides a mechanism to facilitate performing and recording glucose measurements under supervision, while it additionally provides for periodic instrument calibration, and the ability to assure both measurement and calibration compliance. Calibration data can be communicated from instruments in the field
10 to the instrument manufacturer, or a service organization, so that instruments and their calibrations may be logged. The disclosed network link can be utilized to provide various mechanisms for assuring calibration compliance. Generally the mechanisms are of two categories, those that provide information or a warning about calibration, and those that prevent use of an instrument which is out of calibration. Preferably instruments which have exceeded their calibration interval, or schedule, are to be locked out from further use until recalibration is
15 performed. For example, the instrument may be set to operate for thirteen months for a given calibration interval of twelve months. The unit preferably issues warnings prior to the expiration of calibration, and warnings of increased severity after the expiration of the calibration interval. If the unit, however, is not properly calibrated by the end of the thirteen months, normal operation ceases, thereby locking out the user after providing an appropriate error message in regard to the expired calibration. Upon recalibration, the calibrated operation interval
20 is restored to provide for another thirteen month period of calibrated operation.

Alternatively, or in addition thereto, a "lockout command" can be sent to the unit over the communication link from the manufacturer which engages a lockout mode of the device, so that continued operation may not be continued until the unit has been serviced. The lockout command could also be sent in the event that the patient has not paid his or her bills, or be sent under other circumstances warranting lockout of the instrument.

Another mode is that of locking out normal instrument use after the expiration of calibration, and allowing limited use thereafter only after a code, or token, has been downloaded from a supervisory site. Although many variations are possible, the code could for instance be provided when a calibration appointment is made for the instrument. To provide continued service and minimize cost, the patient may be allowed to perform calibration checks of the instrument. The patient is supplied with a small set of glucose calibration standards which are read
30 by the instrument once it is put into a calibration mode and preferable connected to a remote site for supervising the process. Should the calibration check pass, wherein the instrument readings fall within normal levels, or be capable of being automatically adjusted thereto, the calibration interval may be extended. Failure of the calibration check would typically necessitate returning the instrument for service.

FIG. 3 illustrates an embodiment of a process 50 for assuring calibration compliance within the glucose
35 measurement instrument by utilizing a lockout mechanism. The programmed instructions associated with the glucose measurement instrument are started at block 52 and initialized at block 54, and a check is made on a

lockout flag at block 56 to determine if it was set during a prior session by a command received from the internet, or due to being out of calibration. Not having been locked out from a prior session, the real-time clock (RTC) of the device is read at block 58 and a calculation is performed at block 60 comparing the current date with the stored calibration date and calibration interval. If upon checking calibration at block 62 the calibration interval has not yet expired, then a calculation is performed at block 64 comparing the current date with the stored calibration date and near-calibration interval. Near-calibration is checked at block 66 and, if calibration is to expire soon, then a user warning is issued at block 68, preferably informing the user of the date of the upcoming expiration of the calibration interval. The lockout flag is cleared at block 70 and processing within the glucose measurement instrument continues with normal instrument functions being accessible at block 72, along with calibration and other limited functions at block 74, until the user shuts down the instrument and processing ends at block 76. If the lockout flag was set from a prior instrument operation, or the calibration interval was exceeded, then a lockout flag would be set at block 78, and the instrument functionality would thereby be restricted to execution of the calibration procedures and other limited functions at block 78 while the normal instrument functionality would not be accessible. The calibration procedure itself may be augmented and improved by providing interaction between the servicing party and the manufacturer, such interaction may include providing guidance information to the servicing party, and the collection of measurement information by the manufacturer.

It will be appreciated that the present invention provides functionality beyond that which can be provided by a stand-alone glucose measurement instrument, as the practitioner, or practitioners office, is brought back into the glucose measurement process to confer a portion of the benefits normally associated with an office visit. It will be recognized that although not providing equivalent results, the features described for the embodiment of a non-invasive glucose monitoring instrument are generally applicable to glucose monitoring hardware in which invasive measurements are taken. The aforesaid description of the invention illustrates how these features provide the capability for two-way data flow which facilitates the conducting and recording of correct measurements while encouraging compliance in regard to both measurements and instrument calibration. Furthermore, the data collected by the system may be utilized by others in addition to the practitioner, such as pharmaceutical companies which may be provided data access to alter or administer medication programs, and insurance companies which may require data regarding patient diligence according to the specified treatment program.

Accordingly, it will be seen that the present invention provides numerous benefits for patients needing to closely monitor blood glucose levels and it can be implemented with numerous variations and alternatives obvious to those skilled in the art.

Although the description above contains many specificities, these should not be construed as limiting the scope of the invention but as merely providing illustrations of some of the presently preferred embodiments of this invention. Thus the scope of this invention should be determined by the appended claims and their legal equivalents. Therefore, it will be appreciated that the scope of the present invention fully encompasses other embodiments which may become obvious to those skilled in the art, and that the scope of the present invention

is accordingly to be limited by nothing other than the appended claims, in which reference to an element in the singular is not intended to mean "one and only one" unless explicitly so stated, but rather "one or more." All structural, chemical, and functional equivalents to the elements of the above-described preferred embodiment that are known to those of ordinary skill in the art are expressly incorporated herein by reference and are intended to be encompassed by the present claims. Moreover, it is not necessary for a device or method to address each and every problem sought to be solved by the present invention, for it to be encompassed by the present claims. Furthermore, no element, component, or method step in the present disclosure is intended to be dedicated to the public regardless of whether the element, component, or method step is explicitly recited in the claims. No claim element herein is to be construed under the provisions of 35 U.S.C. 112, sixth paragraph, unless the element is expressly recited using the phrase "means for."

WHAT IS CLAIMED IS:

1. In a glucose monitoring instrument having a glucose detection element and signal processing circuitry, the improvement comprising:
 - (a) means for providing network connectivity of the instrument for the communication of
5 measurements and information between a patient and a supervisory authority, such as a medical practitioner; and
 - (b) network protocol associated with said instrument capable of providing said network connectivity.
2. A glucose monitoring instrument, comprising:
 - (a) means for collecting glucose readings from a patient; and
 - (b) means for communicating said glucose readings over a network to a remote station to allow a
10 supervisory authority, such as a medical practitioner, to oversee data collection and patient treatment.
3. In a glucose measurement instrument having means for measuring patient glucose information and processing that information into one or more readings, the improvement comprising:
 - (a) a network communications interface capable of communicating over a connected network; and
 - (b) a controller configured for executing a series of programmed instructions which provide for the
15 communication of measurements and information with a remote station to allow a supervisory authority, such as a medical practitioner, to oversee patient data collection and compliance with a treatment program.
4. In a glucose measurement instrument as recited in claim 3, the improvement further comprising communication of measurement data and time of measurement.
5. In a glucose measurement instrument as recited in claim 3, the improvement further comprising
20 means for communicating a patient initiated alert to said remote station to allow for practitioner intervention on behalf of said patient.
6. In a glucose measurement instrument as recited in claim 4, the improvement further comprising means for communicating calibration information to a remote station to encourage calibration compliance.
7. An apparatus for monitoring the glucose levels of a patient comprising:
 - (a) a glucose detector element capable of taking measurements of bodily glucose levels;
 - (b) a network communications interface; and
 - (c) a processor operating from programmed instructions capable of controlling measurements from
25 the glucose detector element and of communicating glucose measurement information via the network communications interface to a remote location.
8. An apparatus as recited in claim 7, wherein the glucose measurement information comprises
30 measurement data, and time of measurement.
9. An apparatus as recited in claim 7, further comprising means for communicating a patient initiated alert to said remote location to allow for practitioner intervention on behalf of said patient.
10. An apparatus as recited in claim 7, further comprising means for communicating calibration
35 information to a remote station for monitoring and controlling aspects of instrument calibration to encourage calibration compliance.

11. An apparatus as recited in claim 7, wherein the glucose detector performs non-invasive blood glucose measurements.
12. An apparatus as recited in claim 7, wherein the glucose detector element comprises a thermal gradient spectrophotometer.
- 5 13. An apparatus as recited in claim 7, further comprising programmed instructions for the processor to generate alerts to inform the patient when the time arrives for taking a measurement according to a predetermined schedule or network communicated practitioner directive.
14. An apparatus as recited in claim 7, further comprising programmed instructions for the processor to generate warnings transmitted to a remote location when measured glucose levels exceed the bounds of a
10 preselected range.
15. An apparatus as recited in claim 7, further comprising a panic button which upon activation sends an alert to a remote location over the network communication interface to alert a supervisory authority so that intercession on the patients behalf may be instituted.
16. An apparatus as recited in claim 7, wherein periodic maintenance and calibration information
15 is communicated over the network communication interface to a remote location.
17. An apparatus as recited in claim 16, further comprising a lockout mode that is entered upon receiving commands over the network communication interface, the lockout mode restricting the functionality of the instrument which may be accessed by the user.
18. An apparatus as recited in claim 16, further comprising a lockout mode that is entered upon
20 determining that the calibration interval for the instrument has been exceeded, the lockout mode restricting which functions of the instrument which may currently accessed by the user.
19. A method of providing supervision of blood glucose measurements, comprising the steps of:
- (a) collecting a glucose measurement on a portable blood glucose instrument;
- (b) processing the glucose measurement into a set of data values; and
- 25 (c) communicating said data values over a network to a remote station such that a supervisory authority, such as a medical practitioner, may oversee the data collection and patient treatment program, the communication of said data values being provided by a network communications interface within said blood glucose instrument.

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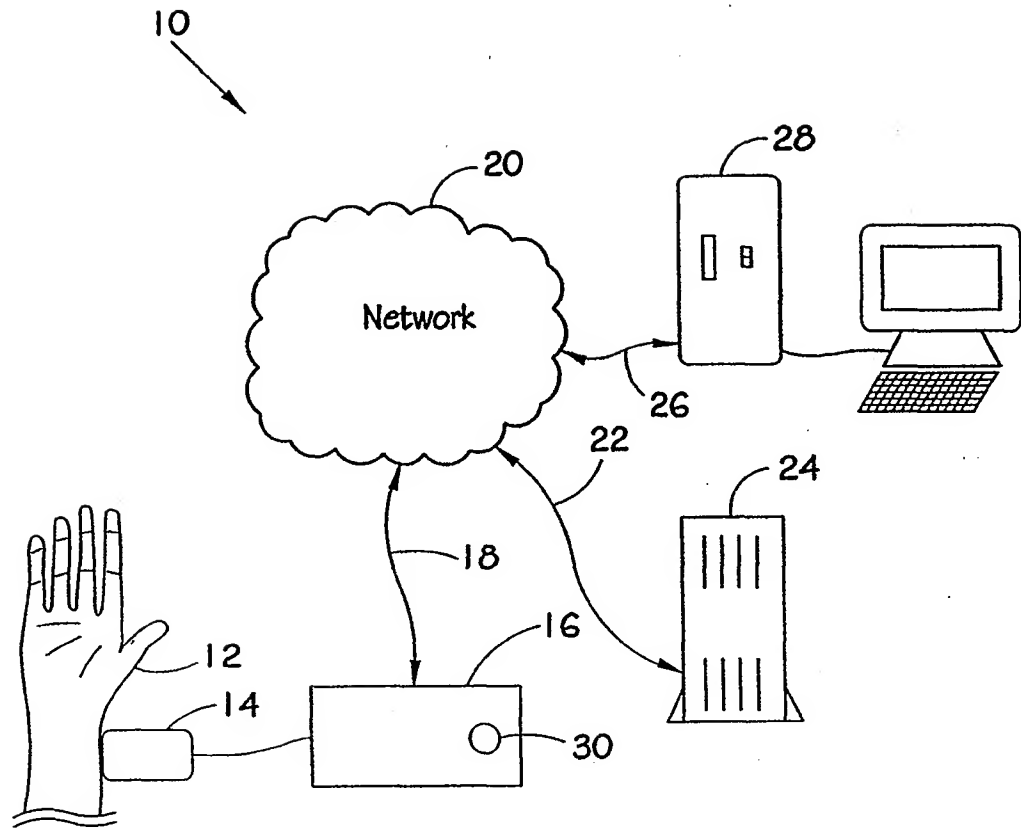


Fig. 1

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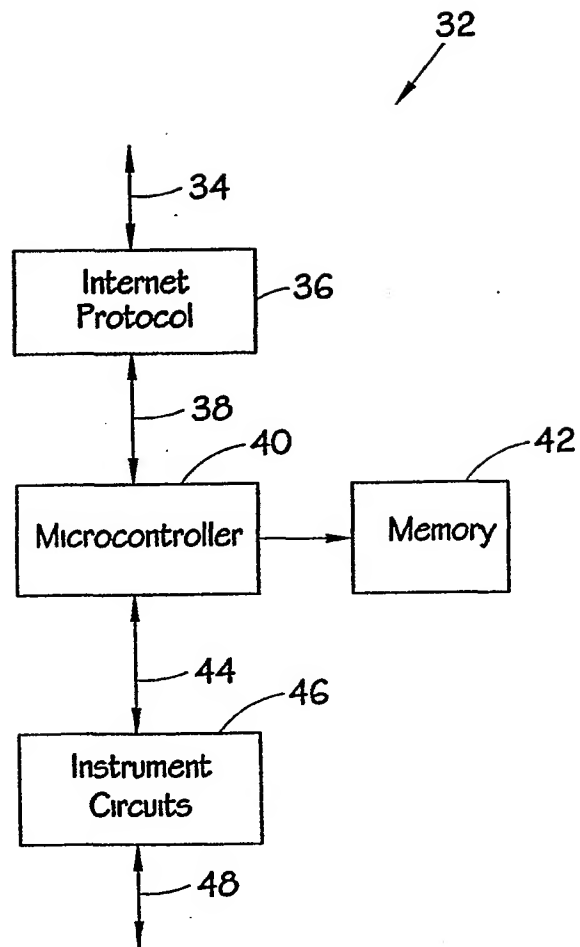
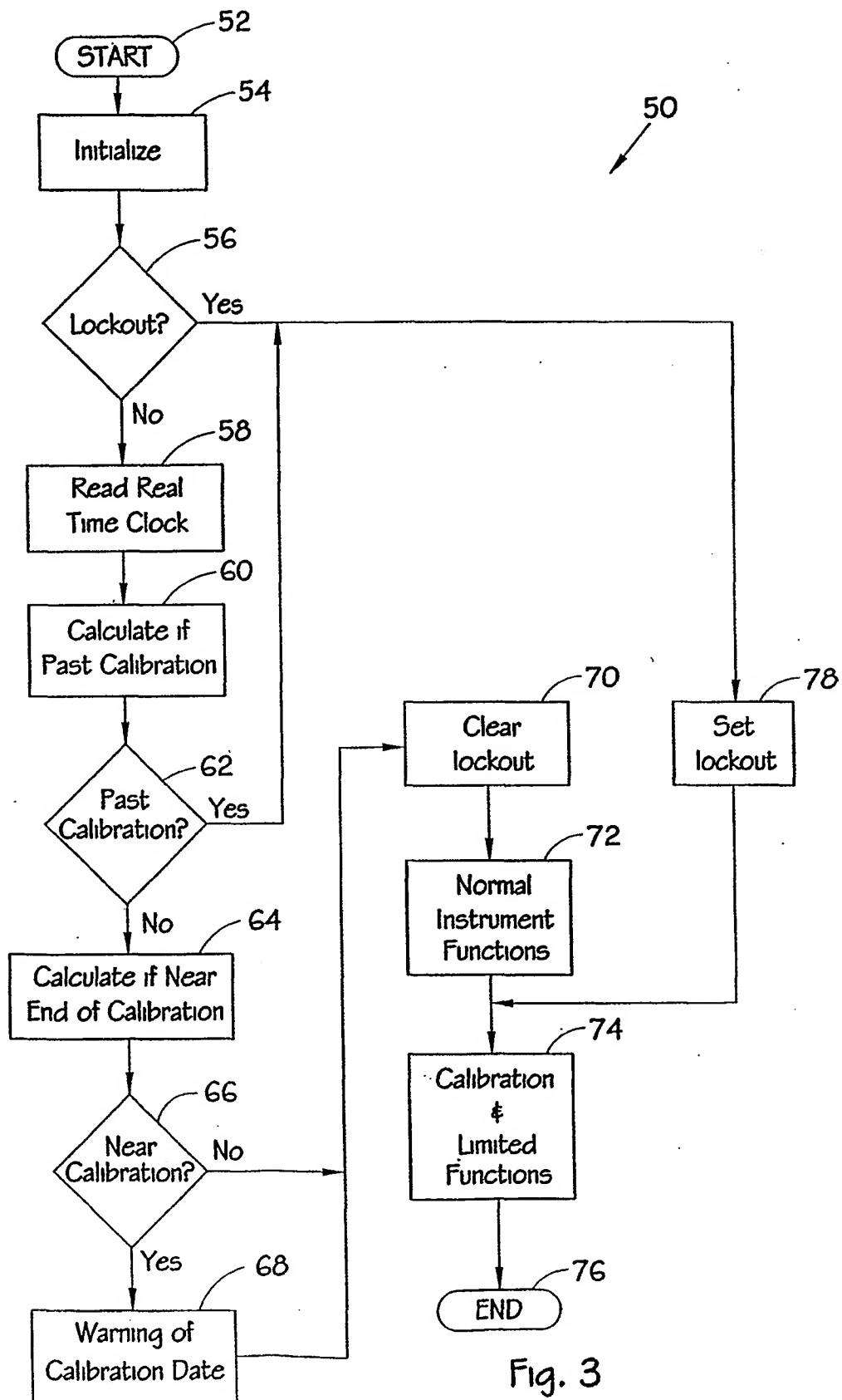


Fig. 2

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INTERNATIONAL SEARCH REPORT

In national Application No
PCT/US 01/46685

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

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Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 28460 A (LIFESTREAM TECHNOLOGIES INC; COAD NOAH M (US); MOODY JAMES L (US);) 18 May 2000 (2000-05-18) page 18, line 4 - line 15	1-4, 6, 7, 10, 16, 19
A	page 21, line 24 - page 22, line 22 ---	11, 12
X	EP 0 970 655 A (CARPE DIEM COMERCIAL SANITARIA) 12 January 2000 (2000-01-12) column 4, line 37 - line 44 column 15, line 20 - line 42 column 20, line 16 - line 21 column 21, line 45 - line 49 column 25, line 40 - line 44 column 30, line 39 - line 43 ---	1-3, 5, 7, 9, 13-15, 19
A	--- -/--	11, 12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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PCT/US 01/46685

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 53085 A (OPTISCAN BIOMEDICAL CORP) 14 September 2000 (2000-09-14) page 11, line 18 - line 20 page 18, line 13 - line 16 page 27, line 18 - line 2 ---	1-3,7, 10-12, 16,19
A		6
X	WO 00 32258 A (AASMUL SOEREN; NOVONORDISK AS (DK); CHRISTENSEN LARS HOFMANN (DK);) 8 June 2000 (2000-06-08) page 7, line 32 -page 8, line 7 page 9, line 8 - line 12 page 12, line 15 - line 20 page 14, line 30 -page 15, line 7 page 32, line 5 - line 11 -----	1-5,7-9, 14,19
A	WO 99 25110 A (I FLOW CORP) 20 May 1999 (1999-05-20) page 4, line 19 - line 34 -----	1-3,5-7, 9,10, 15-19

INTERNATIONAL SEARCH REPORT

In International Application No

PCT/US 01/46685

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0028460	A	18-05-2000	AU 2023100 A EP 1133747 A2 WO 0028460 A2	29-05-2000 19-09-2001 18-05-2000
EP 0970655	A	12-01-2000	ES 2124186 A1 AU 5561698 A BR 9807497 A EP 0970655 A1 CN 1244104 T WO 9831275 A1	16-01-1999 07-08-1998 21-03-2000 12-01-2000 09-02-2000 23-07-1998
WO 0053085	A	14-09-2000	US 6198949 B1 AU 3523000 A EP 1158893 A1 WO 0053085 A1	06-03-2001 28-09-2000 05-12-2001 14-09-2000
WO 0032258	A	08-06-2000	AU 1376500 A AU 1503200 A BR 9916142 A BR 9916149 A CN 1329511 T CN 1329471 T CZ 20011702 A3 CZ 20011815 A3 WO 0032088 A1 WO 0032258 A1 EP 1144028 A1 EP 1135056 A1 NO 20012623 A NO 20012655 A	19-06-2000 19-06-2000 04-09-2001 04-09-2001 02-01-2002 02-01-2002 17-10-2001 16-01-2002 08-06-2000 08-06-2000 17-10-2001 26-09-2001 13-07-2001 25-07-2001
WO 9925110	A	20-05-1999	AU 1584099 A BR 9814637 A CA 2310243 A1 CN 1295754 T EP 1036464 A1 HU 0100368 A2 JP 2001522674 T NO 20002468 A PL 340546 A1 TR 200001711 T2 WO 9925110 A1	31-05-1999 27-11-2001 20-05-1999 16-05-2001 20-09-2000 28-05-2001 20-11-2001 11-07-2000 12-02-2001 22-01-2001 20-05-1999

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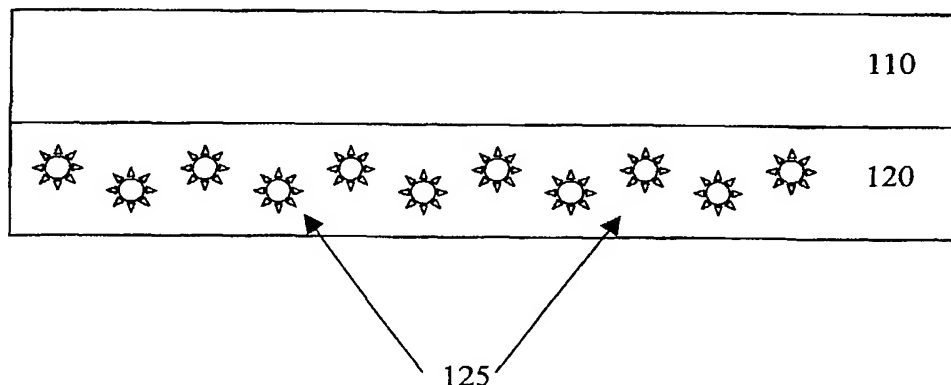
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[Continued on next page]

(54) Title: LAYERED CALIBRATION STANDARD FOR TISSUE SAMPLING

Layered Calibration Device 100



(57) Abstract: The invention relates to fluorescence calibration devices and methods that can mimic skin and other tissues. A calibration device of the invention comprises at least one scattering layer, which is preferably non-fluorescent, and a second layer containing one or more fluorophore. Light passes through the scattering layer and excites the fluorophore. Light emitted from the fluorophore passes back through the scattering layer and into collecting optics, which can be measured and that measurement is used to correct for instrument drift.



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LAYERED CALIBRATION STANDARD FOR TISSUE SAMPLING

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to devices and methods for the measurement of
5 fluorescence spectra from biological tissue. Particularly, the invention relates to a standard for calibrating skin and tissue fluorescence measurement systems.

2. Description of Background

The skin, also known as the integumentary system, is one of the largest
organs in the body. It has a surface area of 1.8 m^2 and makes up approximately
10 sixteen percent of total body weight. As such, the skin represents more than a regulatory and protective barrier, it is a virtual window into the body and can report on a plethora of superficial and/or systemic alterations in health. More recently, the optical diagnostic and interventional potential of *in vivo* fluorescence has also begun to attract interest. Studies involving autofluorescence now include sunscreen testing,
15 as well as laser imaging, diagnostics, drug monitoring and photodynamic therapy. Other specific applications include diagnostic testing for skin pathogens, tumors and blood/interstitial fluid analyte analysis. As a result, understanding light propagation in the skin is now viewed as fundamental to anticipating, assessing, and treating a broad spectrum of normal and abnormal conditions that affect this organ.

20 Optical properties of skin reflect the structure and chemical composition of the skin. When the skin surface is irradiated, part of the energy will be specularly reflected by the surface, while the rest will be refracted and transmitted into the skin. Photons transmitted into the skin will be scattered and absorbed by the skin tissue. After multiple scattering events, some of the transmitted radiation will re-emerge
25 through the air-stratum interface into the air. This re-emergence is called diffuse reflectance. The amount of diffuse reflectance is determined by both the scattering and absorption properties of the skin tissue. Simplistically, the stronger the absorption, the less the diffuse reflection; the stronger the scattering, the larger the diffuse reflectance. Following absorption in the skin, electronically excited
30 molecules can return to a more stable energy state by emitting a photon, which constitutes a fluorescence emission, and the reference molecule is referred to as a

fluorophore. Given that there are native fluorophores localized within skin that are responsible for this fluorescence, this process is also known as autofluorescence.

Fluorescence spectra are quite sensitive to the local molecular environment of the fluorophores. Using fluorescence measurement systems that optically
5 illuminate skin samples over a known range of frequencies and collect and measure the scattered and emitted light, normal human skin has been relatively well characterized. Clearly a complex target, skin has multiple layers, each with distinct fluorescent properties. The epidermis, composed principally of keratinocytes, is the outer, protective, nonvascular layer of the skin. It is subdivided into five layers or
10 strata, the stratum germinatum, the stratum spinosum, the stratum granulosum, the stratum lucidum and the stratum corneum. Histologically, the stratum corneum, the outermost, keratinized layer of dead cells in the epidermis, is the most superficial layer of the skin and provides the first barrier of protection from the invasion of foreign substances into the body. Whereas the deepest epidermal layer, the stratum
15 germinatum, provides the germinal cells necessary for the regeneration of the layers of the epidermis. It is the constant segueing from the live and actively replicating cells of the stratum germinatum to the dead cells of the stratum corneum surface that uniquely characterizes the skin. After a mitotic division a newly formed cell will undergo a progressive maturation called keratinization during which time it migrates
20 slowly to the surface and sloughs off in a process called desquamation. Keratinocytes constitute about ninety five percent of the epidermal cells and function as a barrier, keeping harmful substances out and preventing water and other essential substances from escaping the body. The other five percent of epidermal cells are melanocytes, which manufacture and distribute melanin. Melanin, a large
25 insoluble polymer, is a very complex absorbing material whose complete function is not understood. Generally accepted as an important factor in skin pigmentation, melanin has also been attributed with properties associated with protection from ultraviolet rays. In addition, it is known to scavenge reactive chemical species and metal ions.

The epidermis and dermis are separated by a thin layer of basement membrane to which both layers are attached. The dermis assumes the important functions of thermoregulation and supports the vascular network to supply the avascular epidermis with nutrients. The dermis is typically subdivided into two zones, a papillary dermis and a reticular layer. The dermis contains mostly fibroblasts which are responsible for secreting collagen, elastin and ground substance that give the skin its support and elasticity. Also present are immune cells that are involved in defense against foreign invaders passing through the epidermis. Given the above constituents, the dermis is gel-like and accommodates a variety of embedded structures that are common to other organs such as lymph channels, blood vessels, nerve fibers, and muscle cells, as well as unique structures like hair follicles, sebaceous glands, and sweat glands.

The fundamental principles of the optical properties of human skin is well known in the art, for example, the article published by Hardy *et al.*, entitled "Spectral Transmittance and Reflectance of Excised Human Skin" (Journal of Applied Physics, Vol. 9, pp 257-264, 1956), describes measurements of transmission and remission of an incident beam through skin samples of various thicknesses, including both the epidermis and various amounts of dermis. The study also describes that, as the thickness of the dermis increases, transmission decreases, and becomes more diffuse, suggesting multiple scattering (as described by R. Anderson *et al.*, in "Optical Properties of Human Skin", The Science of Photomedicine, Plenum Press, N.Y., pgs. 147-194, 1982).

Skin or other tissue fluorescence measurements are useful for diagnosing a variety of conditions, and are often used in the cosmetics industry. Fluorescence measurements also are useful to quantitate a concentration of numerous blood analytes. In fluorescence measurement systems, acquisition of fluorescence spectra often involves a fiber optic bundle or other light illumination and collection means, which is pressed against the skin. Generally, an optics illumination/collection device or part provides excitation light to a sample and another part or device collects emission light from the sample. The position and orientation of these

excitation and emission parts or devices can be adjusted to optimize detection of a desired fluorescence signal.

The ability of any signal processing technique to extract information from spectroscopic data for determination of an analyte, for example, glucose concentrations, relies heavily on the processes capability to account for nonlinearities, such a nonlinearities which can result from light penetrating skin at depths greater than 0.5 mm. The studies of Hardy *et al.* and Anderson *et al.* suggest when skin thickness exceeds 0.5 mm, the nonlinear results that must be accounted for and corrected.

Fluorescence measurement systems, therefore, should be well calibrated for the most accurate quantitation. For example, system elements such as excitation source intensity, detector efficiency, and efficiency of the optical train may change over time. Skin light scattering properties also can vary among instruments. These sources of error complicate comparisons of spectra measurements taken at different times. Stable optical calibrators that cover spectral ranges similar to those obtained from test substances are well known. Further, such calibrators exhibit standard fluorescence spectra, allowing the estimation and correction of fluorescence measurement systems. For example, Labsphere (North Sutton, New Hampshire) provides a line of calibration standards for the Spectralon (TM) system.

The layered geometry of the skin is vital to its function and contributes to its characteristic fluorescent spectra. The keratinized stratum corneum is highly scattering to incident irradiation. The deeper epidermal and dermal layers, based on structural elements, the presence of fluorophores and depth, also contribute to the specific spectral profiles identified with human skin. Not unexpectedly, disease processes can contribute to skin changes that are associated with corresponding shifts in structural, chemical, or histological composition. These changes are manifest as well through altered skin autofluorescence and diffuse reflectance patterns. The resulting excitation-emission profiles can also be utilized to analyze and quantify specific blood or interstitial fluid analytes (see PCT/US99/07565, PCT/US01/05323 and any U.S. counterparts). Yet the ultimate accuracy and reliability of such non-invasive optical measurement systems depends on appropriate

calibration schemes that accommodate for source, sample, environmental, and temporal variations. The successful implementation of calibration methodologies is a sophisticated undertaking and requires a full understanding of measurement uncertainty, error, accuracy, precision quality, and reliability. As a rule, the accuracy of a calibration device is directly proportional to the precision of calibration with respect to the intended samples. Given that structural and biochemical factors must be taken into consideration, the design of a calibration system must anticipate skin characteristics in terms of multispectral radiation attenuation, physical morphology, and geometry. For *in vivo* measurement, phantoms have been used to calibrate detection systems. To date, however, available calibration devices have been unable to precisely model the spectral or fluorescence properties attributable to the complexly layered, structurally rich aspects of skin. Conventional calibration devices lack precision and accuracy because they insufficiently imitate the layered, turbid skin medium and the fluorescence properties of that medium. Thus, conventional calibration devices have not been able to characterize instruments intended for scattering, fluorescent, structured targets.

SUMMARY OF THE INVENTION

The invention represents a significant improvement over existing devices and methods and provides accurate calibration devices that simulate any desired material or surface to produce accurate and reliable measurements.

One embodiment of the invention is directed to calibration devices that provide optical and fluorescence properties that simulate those of another material such as biological tissues and fluids. Such devices comprise at least one layer that is composed of or contains a substance of a scattering nature and another layer that contains a fluorophore. Preferably, the device is a fluorescence calibration devices comprising: at least two layers wherein a first layer comprises a scattering material, which is preferably only non-fluorescent or only slightly fluorescent, and a second layer which comprises a material having embedded fluorophores. Preferably, the fluorescence calibration device has fluorescence properties that mimic human skin.

Another embodiment of the invention is directed to methods of correcting for instrumental drift when gathering tissue fluorescence spectra, comprising the steps of: directing excitation light into a calibration artifact containing at least one scattering layer, through the scattering layer and into a fluorophore contained in another layer; exciting the fluorophore; collecting light emitted from the fluorophore; and correcting the instrumental response based on the collected light.

Another embodiment of the invention is directed to methods of calibrating a fluorescence measurement system, comprising the steps of directing excitation light into a calibration device of the invention, which excites the fluorophore and thereby collecting light emitted from the fluorophore; and calibrating the device from a measurement of the collected light.

Another embodiment of the invention is directed to methods of calibrating a fluorescence measurement system, comprising the steps of: determining a calibration target on a fluorescence measuring instrument; exciting the calibration target with amplitude modulated electromagnetic radiation; measuring electromagnetic radiation passing from the calibration target to the fluorescence measuring system; and determining a phase shift between the fluorescing amplitude modulated electromagnetic radiation, wherein the electromagnetic radiation passing from the calibration target to the fluorescence measurement system.

Another embodiment of the invention is directed to calibration devices with spectral characteristics that mimic the fluorescence properties of skin or other tissues.

The foregoing, and other features and advantages of the invention, will be apparent from the following, more particular description of the preferred embodiments of the invention, the accompanying drawings, and the claims.

DESCRIPTION OF THE FIGURE

Figure 1 depicts a calibration device according to one embodiment of the invention.

DESCRIPTION OF THE INVENTION

Skin autofluorescence spectra are quite complex insofar as they are attributable to diverse fluorophores with different lifetimes, spectral properties and

spatial localization. Further, spectrally active components are sensitive to dynamic fluctuations in the concentration of biological analytes. Further still, measurement changes may arise from damage or alterations to the skin, or simple heterogeneity from skin types.

5 It has been surprisingly discovered that an accurate emission spectrum can be created with a calibration device that calibrates multispectral optical sampling of the target being measured to resolve and correct spectral measurements for source, sample, background, environmental, geometric, and temporal variations across a plurality of samples. With devices of the present invention, the accuracy and
10 efficiency of the measurement of optical properties from fluorescence detection systems is significantly increased.

Preferred embodiments of the invention are described with reference to the Figure 1. These preferred embodiments are discussed in the context of calibration devices that mimic human skin and tissue. Nevertheless, the invention can be
15 practiced in the context of layered calibration devices for calibrating a wide variety of fluorescence measurement systems for a variety of purposes, not limited to glucose, and on a variety of tissues.

A layered calibration device according to an embodiment of the invention is illustrated in Figure 1. Layered calibration device 100 comprises top layer 110 and
20 bottom layer 120. Top layer 110 comprises a highly scattering material. Bottom layer 120 comprises a material having embedded fluorophores 125. Top layer 110 and bottom layer 120 have a desired thickness to achieve fluorescence properties that mimic a tissue such as, preferably, human skin, which is well known to those of ordinary skill in the art and can be empirically determined.

25 Embodiments of the invention rely on particles in the top layer to scatter light. These particles may be of a wide range of compositions and sizes. Many polymeric materials form particles of suitable size from 0.1 to 20 microns on average (i.e. at least 95 % of particles falling within this range). In embodiments, narrower ranges are acceptable wherein 90% of particles have mean diameters
30 within the range 0.2 to 1 micron, 0.3 to 1.2 microns, 1 to 20 microns 1 to 5 microns,

0.5 to 5 microns, less than 0.8 microns, or less than 1 microns. Other ranges may be determined based on the particular application by a skilled artisan. In an embodiment the particles have mean diameters that are at least 0.1 times the wavelength of light used. In another embodiment the particles have mean diameters
5 that are at least 0.5 times, 1 time and even 2 times the wavelength of light used. In an embodiment that simulates human skin with cellular material, particles are translucent to visible light and have a mean diameter that is within 0.2 and 2 times the mean wavelength of the light used to determine light scattering.

A wide range of materials may be used for the particles. Barium sulfate is
10 desirable for some embodiments due to its fairly even responsiveness to different wavelengths. Preferably the particles are held in place by a polymeric material. Such particles may be translucent, in which case the polymeric material may have a refractive index that differs and a particle type should be chosen having refractive index values that are at least 0.05, 0.1 or even 0.2 times different than the refractive
15 index of the binder material. Light scattering particles having a refractive index closer to that of the binder refractive index may produce light scattering insufficient to properly simulate the human skin condition. Inorganic particles such as metal oxides typically have a higher refractive index than polymeric materials and are suitable. Other particles described in U.S. Nos. 6,255,027; 5,877,504; 4,981,882;
20 6,156,468 and 4,166,882 represent art known to skilled artisans and are useful.

One embodiment of the invention is directed to a method to calibrate a fluorescence measurement system. Light passes through scattering layer 110 and excites fluorophores 125 buried in a second layer 120. The emitted light then passes back through scattering layer and into the collecting optics of the system. The
25 measured fluorescence calibration spectrum can be used to correct fluorescence instrument measurements based on the amount of light collected. The device can also be used as a standard to compare fluorescence spectra taken at different times and/or on different systems. The preferred device is lightweight, contains no environmentally harmful components, and disposable after a minimum number of
30 uses.

A wide range of fluorophores may be used for embodiments of the invention. Representative fluorescent molecules are available from Molecular Probes (Portland, Oreg.), Eastman Kodak (Huntington, Tenn.), Pierce Chemical Co. (Rockville, MD) and other commercial suppliers known to those of skill in the art. The
5 fluorophore(s) may be conjugated, or may be unconjugated but immobilized within a solid layer such as a polymer. Bimanes, bodipys, and coumarins often are conjugated and are well known, as are fluorescein derivatives. Green-fluorescent Alexa Fluor 488, BODIPY FL and Oregon Green 514 dyes and the red-fluorescent Alexa Fluor 594 and Texas Red dyes, provide extremely bright signals and superior
10 photostability and are advantageous for these reasons. In an embodiment heat stable fluors are preferred such as those described in U.S. No. 5,990,197 issued to Escano et al. In particular, monomeric infrared fluorophores such as described in U.S. Pat. Nos. 5,336,714 and 5,461,136 may be polymerized into, for example, a polyester to
shift their spectral responsivity into the near infrared region. In an embodiment near
15 infrared light of greater than 750 nm and especially greater than 800 nm light is used with a near infrared light absorbing fluorescent molecule for calibration, as near infrared has the ability to penetrate human tissue more easily and is sometimes used.

In addition to or instead of regular fluorescent reporter molecules in the calibration device, inorganic phosphors may be used. A skilled artisan is familiar
20 with a variety of phosphors, that generally are maintained in a dry environment and which provide long decay times. This class of light emitters includes lanthanides as well, such as erbium chelates and the like. The field of semiconductor physics has developed a large number of such substances that generate emission light from excitation radiation. Some of these even act in an anti-stokes fashion, which allows
25 a long wavelength light such as 660 nm or 880 nm light to excite a complex of lanthanide atoms and a shorter wavelength light such as 550 nm is emitted. A skilled artisan in the infrared laser arts is familiar with how to make and use these complexes, as some materials are used in this fashion to visualize infrared laser beams. This kind of emission provides an advantageous embodiment wherein the
30 particles in layer above the light sensitive material affect excitation light differently than the emission light.

Photochemically stable fluorescent molecules are particularly desirable because of the need for reproducibility between measurements. The term "photochemically stable" in this context means that after repeated exposure a similar response can be obtained. In embodiments, the amount of radiation that is re-emitted when exposed to a constant energy source having an intensity of normal room lighting does not vary by more than five percent after at least 100, 250, 500, 1000, and even 5000 exposures. In embodiments a single exposure has a duration of 0.2 seconds, 1 second, 10 seconds and one minute.

Another embodiment of the invention is directed to a method comprising the steps: (i) directing excitation light into a calibration target containing at least one scattering layer, through the scattering layer and into a fluorophore contained in another layer; (ii) exciting the fluorophore; (iii) collecting light emitted from the fluorophore; and (iv) correcting the instrumental response based on the collected light.

Another embodiment of the invention is directed to a method of calibrating a fluorescence measurement system comprising the steps: (i) determining a calibration target on a fluorescence measuring instrument; (ii) exciting the calibration target with amplitude modulated electromagnetic radiation; (iii) measuring electromagnetic radiation passing from the calibration target to the fluorescence measuring system; and (iv) determining a phase shift between the fluorescing amplitude modulated electromagnetic radiation and the electromagnetic radiation passing from the calibration target to the fluorescence measurement system.

The term "modulated electromagnetic radiation" means that the amplitude and/or the frequency of the radiation is controlled in a reproducible way. In most embodiments the amplitude is controlled with a time varying (usually sinusoidal) signal. For example, a light emitting diode power circuit voltage may be altered to modulate the strength of the emitted light. Demodulation occurs by converting the modulation information back into a signal without the carrier light. A phase shift may be determined by sensing a time difference between the modulation frequency and the demodulated frequency. If an emission signal from a fluorophore is delayed

10 nanoseconds then the demodulated frequency, when compared to the modulating frequency will be delayed by that amount. In an embodiment an inorganic phosphor is used to generate a longer delay of at least 0.5, 1, 2 5 or even 10 milliseconds to provide larger time differences, and lower modulation frequencies. Use of lower
5 modulation frequencies, such as less than 100 megahertz, 10 megahertz, 1 megahertz or even less than 100 kilohertz made possible by use of inorganic phosphors is desirable to keep the equipment complexity and cost down. The cost further minimized by the use of long wavelength light (greater than 600, 720, 760 or even 800 nm) generated by a photodiode or diode laser. A charge coupled device
10 (CCD) or other two dimensional imaging device may be used as is known in the photoimaging art.

Another embodiment of the invention is directed to a method of calibrating a fluorescence measurement system, which can be applied in a technique for detecting cancer and precancerous conditions in skin, tissues and/or cells, wherein the system
15 employs native fluorescence excitation spectroscopy.

Another embodiment of the invention is directed to a method of calibrating a fluorescence measurement system, in which the native fluorescence excitation spectra is measured at 340 nm emission with excitation over the 250 nm to 320 nm spectral region, for malignant tissues and cells are distinguishable from the
20 corresponding excitation spectra for normal tissues and cells. Fluorescence properties are adjustable, for example, by adjusting to a desired thickness, fluorophore color (e.g. white, blue), type, concentration or distribution, fluorophore particle size, device or layer shape, or combination thereof. Alternatively or in addition, a carrier such as a clear or colored matrix or polymer can be used to adjust
25 fluorescence properties. In a preferred embodiment, fluorescence properties are adjustable through a wavelength ranging of 200 nm to 1000 nm, and can be matched to mimic specific tissues, fluids or organs such as human skin.

Another embodiment of the invention is directed to a method of calibrating a fluorescence measurement system that provides support to a technique for detecting
30 the presence of cancer-related, mutant proteins in samples, such as tissue samples and/or cell samples. This method can be applied to various tissues, including tissues

from a part of the body, but not limited to, arteries, bladder, blood, brain, breast, capillary beds, cervix, colon, cornea, eye retina, gastrointestinal tract, gynecological tract, hair, heart, intestines, kidney, liver, lung, muscle, ovary, prostate, retinal blood vessel, skin, stomach, tumor, veins, and combinations thereof.

5 Another embodiment of the invention is directed to a layered calibration device that can be utilized non-invasively for calibrating sampling optics (e.g. U.S. Patent Nos. 6,205,354, and 6,088,087), used for measuring blood volume and analyte concentration and for obtaining spectroscopic information relating to immobile tissues, such as skin. The invention provides a noninvasive calibration
10 device for sampling optics used for determining concentration of an analyte in blood of a subject. Examples of an analyte include, but are not limited to, glucose, urea, total protein, free fatty acids, monoglycerides, diglycerides, triglycerides, creatinine, exchangeable protein associated amide protons, nucleic acids, cholesterol or combinations thereof.

15 Another embodiment of the invention is directed to a layered calibration device that can be used in calibrating a system for determining cell and/or organ function by measuring the blood pool clearance of a targeted agent, referred to herein as tracer (see U.S. Patent No. 6,228,344). The cell and/or organ function can be determined by the rate these cells remove the tracer from the bloodstream.
20 Function can also be assessed by measuring the rate the cells of interest accumulate the tracer or convert it into an active or other form. The agent, which may contain a chromophore and/or a fluorophore, may be targeted to a group of cells or organ which is a high capacity clearance system.

For agents containing chromophores and/or fluorophores, blood pool
25 clearance is measured using a light source/photocell device that measures tissue absorbance or fluorescence in a non-target site, such as an ear lobe, finger, brain or retina. Accumulation of the tracer within the cells of interest is assessed in a similar fashion. The detection of such accumulation is facilitated by using fluorophores which emit in the near infrared wavelengths since body tissues are relatively
30 transparent at these wavelengths.

The agent may be introduced into the patient by any suitable method, including intravenous, intraperitoneal or subcutaneous injection or infusion, oral administration, transdermal absorption through the skin, or by inhalation.

The present invention also can be used for calibrating a system used for the
5 rapid bedside evaluation of biologic functions (see U.S. Patent No. 6,228,344). For example, data on cardiac output, cause of hypercholesterolemia, as well as renal and hepatic function, may be obtained in less than sixty minutes at the bedside after a single intravenous injection. In accordance with one embodiment, a patient may receive a bolus injection of a plurality (e.g. 3, 4, 5, 6, etc.) of different compounds,
10 each containing a different agent (e.g. a fluorophore).

The layered calibration device of the present invention also can be used to support system used for fluorescence detection of an agent which is cleared from the bloodstream by the kidneys or liver. Calibration of assessment of renal or hepatic function by *in vivo* fluorescence detection is encompassed within the invention. The
15 invention can also be used to calibrate the monitoring of the efficiency of hemodialysis. Tumor cells or brain cells also can be targeted in accordance with the invention.

The clearance of tracers can be determined simultaneously by selecting excitation wavelengths and filters for the emitted photons. The concentration/time
20 curves may be analyzed in real time by a microprocessor with any resulting clearance rates calculated and displayed for immediate clinical impact. In cases where unlabeled competing compounds are present (e.g. LDL, asialoglycoproteins), a single blood sample may be analyzed for the concentration of these competing compounds and the results used to calculate a flux (micromoles/minute) through the
25 clearance pathways.

Via linear combination analysis, which is well known to those of ordinary skill in the art, calibration procedures can be implemented that interpret digitized spectra that have been subjected to mathematical algorithms and recorded as pixels. Hence, the layered calibration device of the present invention can be used to support
30 spectral bio-imaging methods (see U.S. Patent No. 5,784,162). The device also can be used, for example, biological research, medical diagnostics and therapeutics. The

imaging methods in the are used to detect spatial organization (*i.e.*, distribution) and to quantify cellular and tissue natural constituents, structures, organelles and administered components such as tagging probes (*e.g.*, fluorescent probes) and drugs using light transmission, reflection, scattering and fluorescence emission strategies, with high sensitivity and high spatial and spectral resolutions.

The layered calibration device described herein can be used to calibrate a method and an apparatus for detecting the presence of a cancerous tissue, such as disclosed in U.S. Patent No. 5,687,730. This U.S. Patent relates to a method and apparatus for detecting the presence of cancerous tissue using fluorescence. The publication relates to an apparatus for detecting the presence of abnormal tissue within a target tissue beneath the skin of a patient containing a light source producing excitation light and a calibration means.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein.

CLAIMS

1. A spectral calibration device comprising:
a first layer comprising an optical scattering material; and
a second layer comprising a fluorescent agent that emits radiation
5 when subjected to an energy source,
wherein fluorescence emitted from said device provides calibration
information for a spectral measurement system.
2. The device of claim 1 wherein the optical scattering material
comprises particles.
- 10 3. The device of claim 2 wherein the particles comprise barium sulfate
4. The device of claim 2 wherein optical scattering of said optical
scattering material can be varied by altering particle size.
5. The device of claim 1 wherein optical scattering of said optical
scattering material is similar to optical scattering of a target of said spectral
15 measurement system.
6. The device of claim 5 wherein the target is skin.
7. The device of claim 1 wherein the radiation emitted by the device is
between about 200 nm and about 800 nm.
8. The device of claim 1 wherein the radiation emitted by the device is
20 between about 300 nm and about 500 nm.
9. The device of claim 1 wherein the energy source is visible light.
10. The device of claim 1 wherein the fluorophore is photochemically-
stable.
11. The device of claim 10 wherein the photochemically stable
25 fluorophore is a fluorophore that emits an amount of radiation when exposed to a

constant energy source wherein said amount does not vary by more than five percent after at least 250 exposures to said source.

12. The device of claim 10 wherein the photochemically stable fluorophore is an inorganic phosphor.

5 13. The device of claim 1 wherein the first layer is proximal to said energy source and the second layer is distal to said energy source, such that radiation passes through said first layer before reaching the agent.

14. The device of claim 1 wherein the spectral measurement system provides a glucose level determination for a patient.

10 15. A method of calibrating a spectral measurement comprising:
directing excitation radiation to a calibration device comprising at least one optical scattering layer and at least one other layer containing at least one fluorophore, wherein optical radiation emitted from said device provides calibration information;

15 collecting spectral radiation emitted from the device; and
calibrating the spectral measurement with the calibration information collected.

16. The method of claim 15 wherein the spectral measurement is fluorescence.

20 17. The method of claim 15 wherein the spectral measurement is indicative of a glucose level of a patient.

18. The method of claim 15 wherein the excitation radiation comprises visible light.

25 19. The method of claim 15 wherein the scattering layer comprises particles of barium sulfate.

20. The method of claim 15 wherein the fluorophore comprises a photochemically stable inorganic phosphor.

21. The method of claim 15 wherein the spectral radiation emitted is between about 200 nm and 800 nm.

5 22. The method of claim 15 wherein the spectral radiation emitted is between about 300 nm and 500 nm.

23. The method of claim 15 wherein the emitted radiation is collected with a CCD camera.

24. The method of claim 15 wherein the calibration information is
10 determined from the amount of radiation detected from the fluorophore.

25. A method of calibrating a fluorescence measurement comprising:
selecting a calibration target on a fluorescence measuring instrument;
exciting the calibration target with modulated electromagnetic
radiation;
15 detecting spectral radiation emitted from the calibration target; and
demodulating the spectral radiation detected to generate a difference
signal that is indicative of a phase shift between the modulated electromagnetic
radiation and the spectral radiation emitted from the calibration target.

26. The method of claim 25 wherein the calibration target is selected
20 from the group consisting of tissues of arteries, bladder, blood, brain, breast,
capillary beds, cervix, colon, cornea, eye retina, gastrointestinal tract, gynecological
tract, hair, heart, intestines, kidney, liver, lung, muscle, ovary, prostate, retinal blood
vessel, skin, stomach, tumor, veins, and combinations thereof

27. A method of correcting for drift of an optical instrument comprising:
directing excitation radiation to a calibration device comprising at
least one scattering layer and at least one other layer that contains a fluorophore;

5 collecting emitted radiation from the device and determining a
correction signal; and

calibrating the optical instrument from the correction signal.

28. A device for calibrating a glucose level detection instrument
comprising an outer layer containing an optically scattering material and an inner
10 layer comprising a fluorophore, wherein visible radiation emitted through said outer
layer impacts said fluorophore which thereby fluoresces and provides a calibration
correction measurement to the glucose level detection instrument which provides an
accurate glucose level determination for a patient.

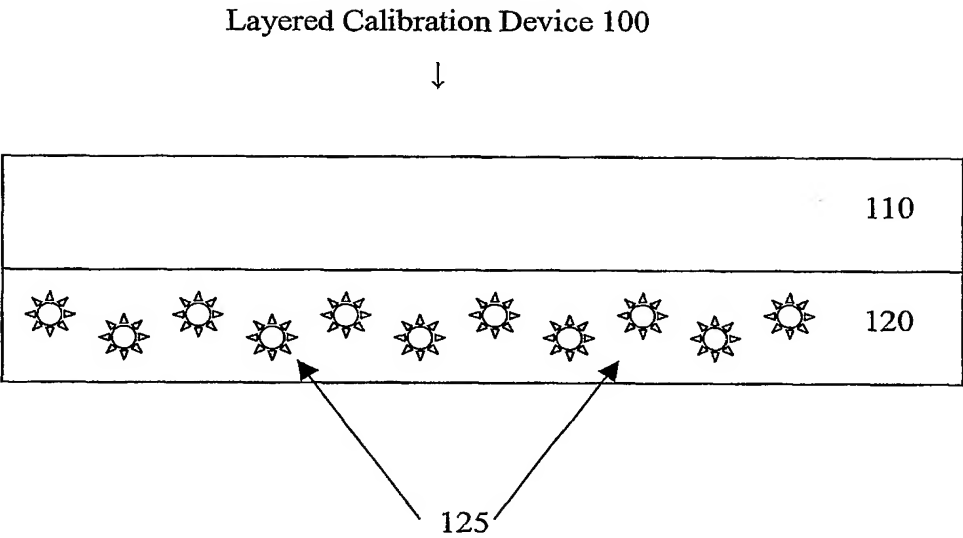


Figure 1

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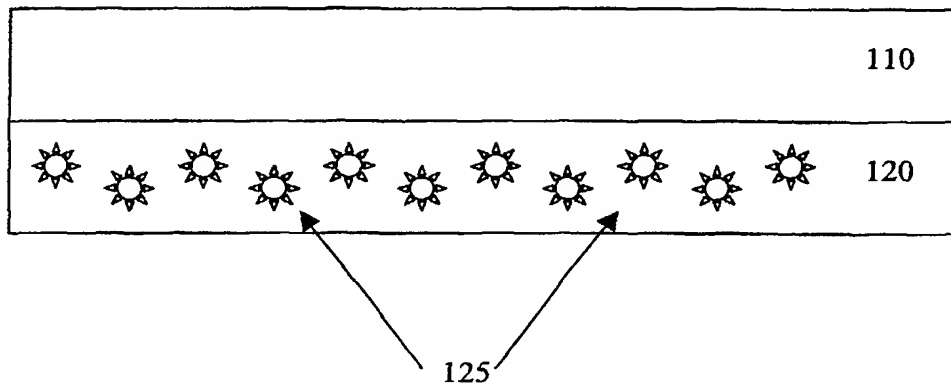
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(54) Title: LAYERED CALIBRATION STANDARD FOR TISSUE SAMPLING

Layered Calibration Device 100



(57) Abstract: The invention relates to fluorescence calibration devices and methods that can mimic skin and other tissues. A calibration device (100) of the invention comprises at least one scattering layer (110), which is preferably non-fluorescent, and a second layer (120) containing one or more fluorophore (125). Light passes through the scattering layer (110) and excites the fluorophore (125). Light emitted from the fluorophore (125) passes back through the scattering layer (110) and into collecting optics, which can be measured and that measurement is used to correct for instrument drift.

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U.S. : 356/317, 318, 417, 243.1, 2435; 250/252.1, 458.1, 459.1, 461.1, 461.2

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NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST search terms: skin, autofluorescence, standard, calibrat\$, scatter\$

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,002,482 A (ROTHFRITZ et al.) 14 December 1999 (14.12.1999), column 11, lines 55-67 and column 12, lines 1-8.	25,26

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

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